GABA and glutamate mimetics

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GABA AND GLUTAMATE MIMETICS

Submitted by

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To my parents and the memory of my dear brother

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SUMMARY

The major objectives of this study were to synthesise new aziridinyl and aminomethyl-γ-lactone mimetics of GABA and to a lesser extent to investigate improved routes to the β-lactam component of Tabtoxin, a glutamine synthetase inhibitor.

Both R- and S-isomers of 5-aminomethyl butyrolactones and (2-aziridinyl)-3-propanoic acids have been synthesised from L- and D-glutamic acids. The lactones were prepared from the known 5-azidomethyl butyrolactone. Hydrolysis and esterification of this lactone afforded the 2-azido alcohol which was converted into the novel (2-aziridinyl)-3-propanoic acid. (R,S)-4-Aminomethyl butyrolactone was prepared from (R,S)-paraconic acid *via* the known 4-hydroxymethyl butyrolactone.

(2-Aziridinyl)ethanol, a precursor of 2-aziridinyl acetic acid has been synthesised from 3-buten-1-ol. 4-Azido-3-iodobutyl acetate and 3-chloro-4-ethoxyformamidobutyl acetate derived from 3-buten-1-ol afforded (2-aziridinyl)-ethanol when treated with LAH and potassium hydroxide respectively.

In the receptor binding tests, R-(-)-5-aminomethyl butyrolactone was found to be as effective as GABA at displacing [^{3}H]-muscimol from the GABA receptor, while the corresponding (R)-(+)-(2-aziridinyl)-3-propanoic acid was active at the concentration of 400 μM.

Deprotection of Tabtoxinine-β-lactam precursors prepared from L-glutamic acid by utilising the Passerini reaction has been investigated. (S)-(+)-Methyl-6-dimethoxyphosphoryl-5-oxo-2-trifluoroacetylaminohexanoate has also been prepared by the reaction of trimethyl phosphite with an appropriate α-chloroketone derived from L-glutamic acid.

NOMENCLATURE

In the text, numbers in parentheses refer to diagrams of formulae and the Arabic superscripts indicate references to the bibliography. The following abbreviations occur in the text:

AIBN	2,2'-azobisisobutyronitrile
Ac	acetate
t-Bu	*tertiary*-butyl
BMS	borane-methyl-sulphide complex
BOC	*t*-butyloxycarbonyl or carbo-*t*-butoxy
BTEAC	benzyltriethylammonium chloride
m-CPBA or MCPBA	*m*-chloroperoxybenzoic acid
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DCU	*N*,*N*-dichlorourethane
DCC	dicyclohexylcarbodi-imide
DMAP	dimethylaminopyridine
DEAD	diethyl azodicarboxylate
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulphoxide
Et	ethyl
HMPA	hexamethylphosphoramide
HPLC	high pressure liquid chromatography
IR	infrared
GABA	γ-aminobutyric acid
LAH	lithium aluminium hydride
LDA	lithium di-isopropylamide
Me	methyl
m/z	mass to charge ratio
mmol	millimole
m.p.	melting point
M.S.	mass spectrum
MsCl	methanesulphonyl chloride
NBS	*N*-bromosuccinimide
N.M.R.	nuclear magnetic resonance

Ph	phenyl
Pht	phthalimido
Py	pyridine
PCC	pyridinium chlorochromate
R_f	retention index for thin layer chromatography
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMSI	trimethylsilyl iodide
TsCl	tosyl or *p*-toluenesulphonyl chloride
UV	ultra-violet
Δ	heat or reflux
Z	benzyloxycarbonyl (or carbobenzoxy)

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PART A

INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION : GABA AND ITS ANALOGUES

1.1 GABA and Inhibition

γ-Aminobutyric acid (GABA) (1) was identified as an important brain constituent only in the 1950's,[1,2] probably because it was not found in any signficant amount in other tissues, and its presence was therefore unexpected. Its functional role as a possible inhibitory transmitter became apparent when Elliot and his collaborators[3,4] identified GABA as the active agent in brain extracts which had a unique inhibitory action on crayfish stretch-receptors.[5,6] The inhibitory effect of GABA on crustacean stretch-receptors and muscles was later shown to be indistinguishable from synaptic inhibition.[7,8] With the further demonstration that GABA is selectively released by stimulating inhibitory nerve fibres,[9] it became probable that it is an inhibitory transmitter in crustaceans.

Hayashi[10] first noted that GABA and some other omega mono-carboxylic amino-acids inhibit mammalian cortical neurones. A depressant action was also observed by Purpura *et al.*[11] and Curtis and his collaborators,[12,13] but neither group believed that GABA could be responsible for the natural inhibition of central neurones. Further tests on the cerebral and cerebellar cortex[14] revealed such a powerful inhibitory effect that GABA could not be ignored as a possible inhibitory transmitter. Later studies[15,16] demonstrated a remarkable similarity between the effects produced by synaptic inhibition and by GABA.

Comparable observations on neurones in Deiter's nucleus make it probable that GABA is the inhibitory transmitter released in the medula by endings in the cerebellar purkinje cells.[17]

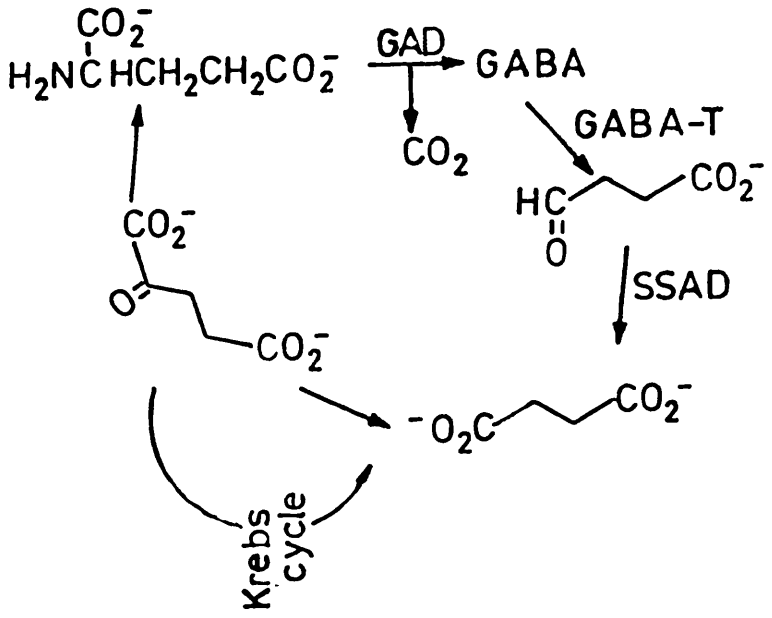
1.1.1 Metabolism and Location

GABA is widely distributed in the brain[18] and is present in substantially higher concentrations (2-4 μmol/g of brain) than many other neurotransmitters.[19] It is also present in some peripheral tissues and is particularly high in concentration in reproductive organs.[20] GABA is also found in glial cells in the central nervous system. Glial cells, like GABA neurons have the capacity for high affinity uptake of GABA, and can release GABA when exposed to a sufficiently high external concentration of potassium ions. Glial GABA accounts for approximately 50% of the GABA in the brain.

The biosynthesis of GABA occurs only in the neurones, since it cannot penetrate the blood-brain barrier, and no peripheral precursor is known. Its production from glutamic acid is catalysed by a highly specific enzyme, glutamic acid decarboxylase (GAD) which requires pyridoxal phosphate (vitamin B_6) as coenzyme. GAD and GABA have an approximately similar distribution in the central nervous tissue. The enzyme is not found in peripheral nerves or spinal roots and its concentration is greatest in areas of the brain stem that are exceptionally rich in GABA (such as the *Substantia nigra*). It is also found in the retina.

Since the production of GABA is an irreversible reaction and GAD is the rate-determining enzyme, GABA metabolism can be regulated by the manipulation of this enzyme or pyridoxal or both (Scheme 1).

GABA can be deactivated and recycled by the transamination with α-ketoglutarate to yield glutamate. This process is catalysed by an enzyme called γ-aminobutyric acid-α-ketoglutarate transaminase (GABA-T). By reversible transamination with α-oxoglutarate, this enzyme converts GABA to succinic semialdehyde, which, in the presence of an appropriate enzyme called succinic semialdehyde dehydrogenase (SSAD), is oxidised to succinic acid. Because succinic acid is a component of the tricarboxylic acid cycle, GABA is conveniently removed by this route.



Scheme 1

GABA-T is also present in the brain, but its distribution differs somewhat from that of GABA.[21,22] It seems that the concentration of

GABA in a given region is determined by local GAD, rather than GABA-T activity.[21] Glucose from the external neuron is the fundamental precursor of GABA.

Significant reduction in the concentration of GABA by inhibition of the decarboxylase markedly increases the susceptibility of animals to convulsive seizures.[23] On the other hand, inhibition of the relevant transaminase (GABA-T) protects GABA against degradation and may therefore promote neuronal inhibition. As a consequence, numerous inhibitors of GABA-T have received attention as potential anticonvulsant agents.[24]

1.1.2 Action of GABA

GABA inhibits neurotransmission by increasing cell membrane permeability to chloride ions. Two types of inhibition have been identified.[20b] In the first type the release of GABA from a neuron terminal partially depolarises the terminal of an excitatory neuron. The partial depolarisation causes a reduction in the release of the excitatory transmitter. This mode of inhibition is called presynaptic inhibition. The second, more classical mechanism of inhibition occurs at synapses between GABA neuron terminals and cell bodies. GABA hyperpolarises the same and/or dendrites of the next cell in line, giving rise to a higher than normal potential gradient. Since this excessive charge differential cannot be compensated for, the neuron is unable to fire.

1.2. GABA Analogues

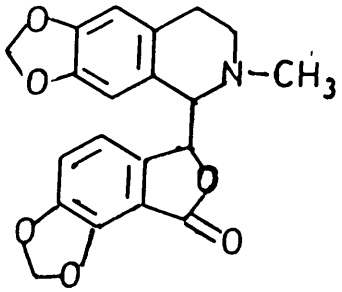
The GABA molecule (1) has considerable flexibility as a result of free rotation around the single bonds, as illustrated in Figure 1. This conformational mobility can be reduced in analogues of GABA by

(I)

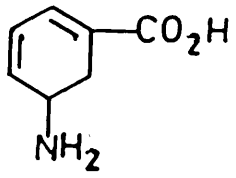
Figure 1

incorporation of unsaturation, ring structures, or both, into the basic transmitter molecule. Such conformational restriction usually results in compounds having more selective actions than that of the transmitter itself, or in having no action at all on GABA processes. Substituents can also produce restriction of otherwise relatively free rotation around single bonds.

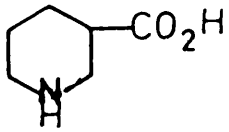
GABA analogues of restricted conformations are well documented.[25] Both natural and synthetic analogues have been investigated. Among the naturally occurring GABA analogues[20b] known are muscimol, a GABA agonist, bicuculline, a reversible antagonist of GABA, gabaculine, a potent irreversible inhibitor of GABA-T and nipecotic acid, a GABA uptake inhibitor.



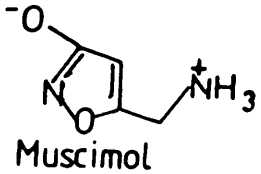
Bicuculline



Gabaculine



Nipecotic Acid



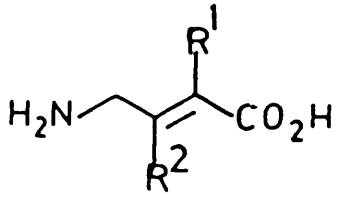
Muscimol

1.2.1 Simple Synthetic GABA Analogues

A variety of compounds both of simple and complex structures have been designed and synthesised as GABA analogues and tested for their biological activity by different groups of researchers. The following review discusses some of the syntheses carried out by some of the researchers.

A R.D. Allan and co-workers

Allan *et al.*[26] have synthesised some substituted 4-aminobut-2-enoic acids (2) as analogues of the neurotransmitter GABA. These compounds were prepared by allylic bromination of a series of α,β-unsaturated acids with *N*-bromosuccinimide to give crude products



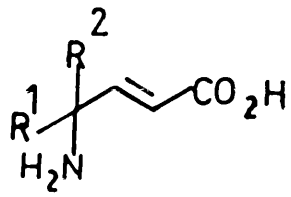
(2)

	R^1	R^2
(a)	H	H
(b)	H	CH_3
(c)	CH_3	H
(d)	Cl	H
(e)	Br	H
(f)	H	Br

which were aminated in liquid ammonia to yield structures 2(a)-(f). These are GABA analogues of restricted conformation with restricted rotation around the C3-C2 bond. Biological activity was observed for some of the compounds.

4-Alkyl-4-aminobut-2-enoic acids[27] have been prepared as conformationally restricted analogues of GABA. The synthetic route involved allylic bromination of α,β-unsaturated acids, followed by

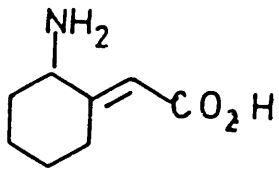
displacement with ammonia. Biological activity was observed in the case of (3), while (4) and (5) were inactive.



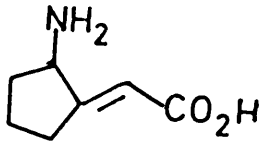
(3)

(a) $R^1 = CH_3$, $R^2 = H$

(b) $R^1 = CH_3 = R^2$



(4)

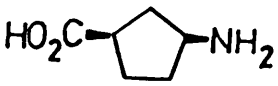


(5)

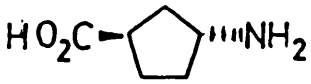
All four stereoisomers of 3-aminocyclopentanecarboxylic acid (7)-(10)[28] were synthesised. The preparation of these acids was based on the Schmidt reaction on the appropriate optically pure keto acids (6) by methods whereby the reaction products were purified directly by crystallisation. These four cyclopentane analogues of GABA were found to have stereoselective actions on the sodium-independent bindings of GABA to rat brain membranes and the sodium-dependent uptake of GABA by rat brain slices.

The three unsaturated derivatives of 3-aminocyclopentane-1-carboxylic acid[29] were also synthesised as conformationally restricted analogues of GABA. Compound (11) was prepared by the Diels-Alder addition of tosyl nitrile to cyclopentadiene which gave the intermediate (14), which yielded (11) after acid hydrolysis. Isomerisation of (11) with 2 M sodium hydroxide gave the conjugated isomer (12).

(6)



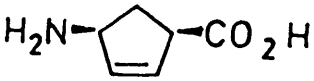
(7) 1R, 3S



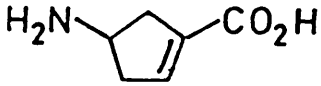
(8) 1R, 3R

(9) 1S, 3R

(10) 1S, 3S



(11)



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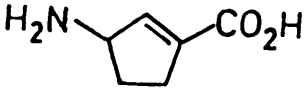
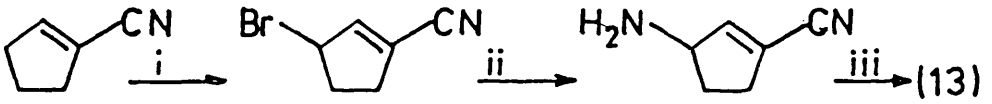


(13)

(14)

The amino acid (13) was prepared from cyclopent-1-ene-1-carbonitrile (15). Reaction of (15) with *N*-bromosuccinimide gave (16) and another product from the alternative allylic bromination. Amination of the crude mixture with liquid ammonia at -60 to -70 °C gave the crude product (17). Acidic hydrolysis of (17) yielded the amino acid (13) (Scheme 2). The introduction of a double bond into the cyclopentane ring as an extra conformational constraint reduced the flexibility of such derivatives.

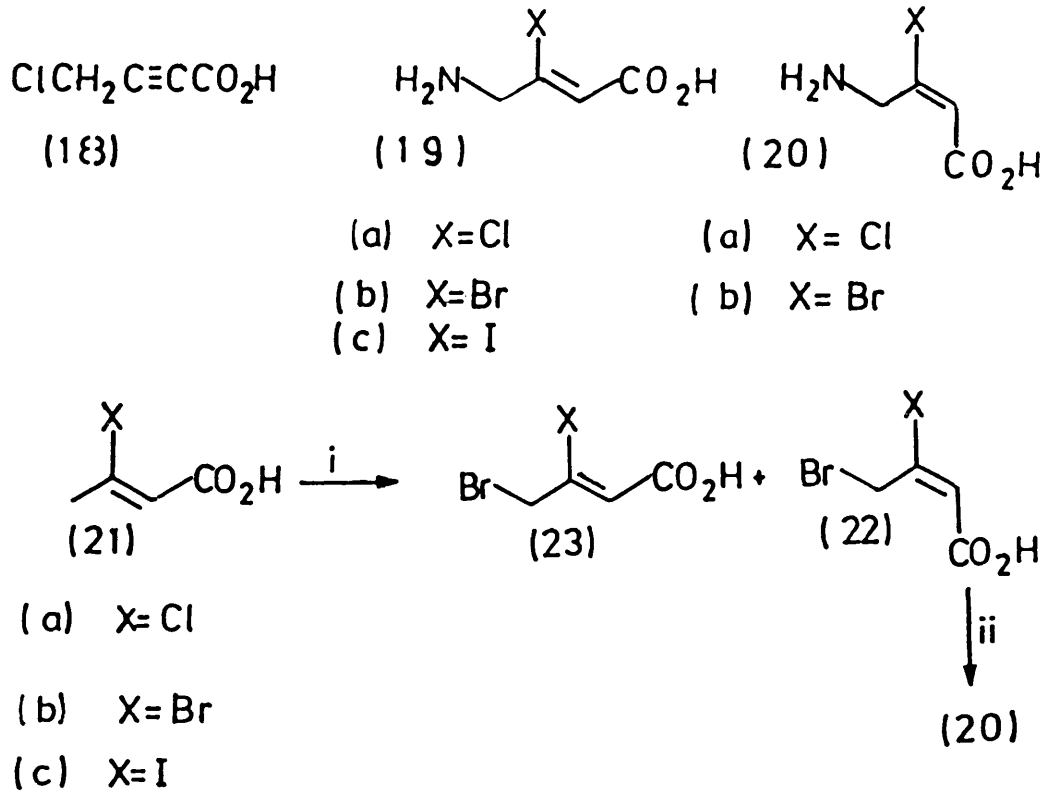


(15) (16) (17)

Reagents: i, NBS / CCl_4 ; ii, Liq. NH_3 / Et_2O; iii, Conc. HCl.

Scheme 2

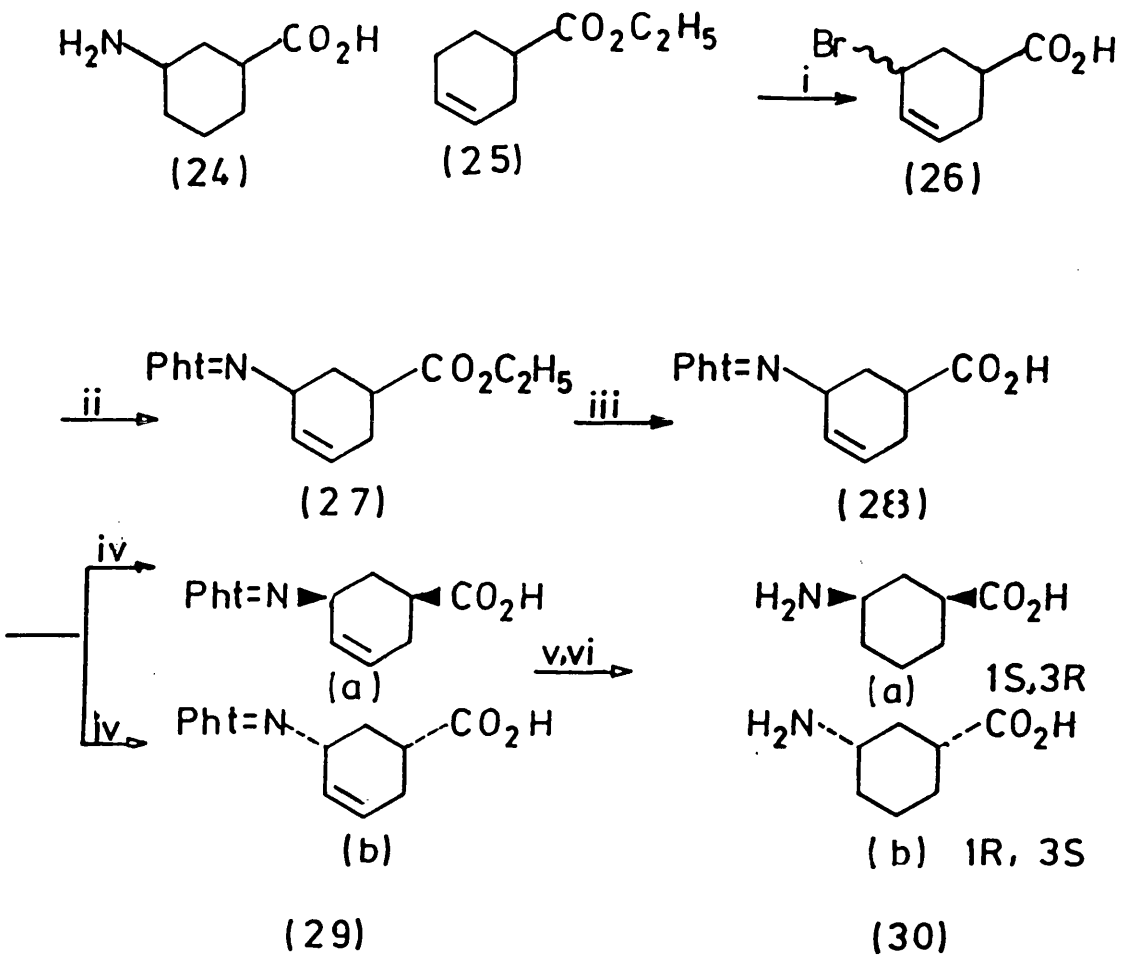
Both *trans*- and *cis*-isomers of 4-amino-3-halogenobut-2-enoic acid[30] were also synthesised as potential irreversible inhibitors of GABA-T. The *trans*- isomers (19) were synthesised by *trans*- addition of HX to 4-chlorobut-2-ynoic acid (18), followed by amination with ammonia, while the key step in the synthesis of the *cis*- isomers (20) was the isomerisation to *cis*-4-bromo-3-halogenobut-2-enoic acids (22) during allylic bromination of (21). Amination of (22) with liquid ammonia furnished the *cis*- isomers (20).



Reagents: i. NBS/CCl_4/AIBN; ii. Liq. NH_3/Et_2O

Scheme 3

In continuation of the search for GABA analogues with restricted conformation, stereoisomers of *cis*-3-aminocyclohexanecarboxylic acid (24)[31] were synthesised. Thus allylic bromination of ethyl cyclohex-3-ene-1-carboxylate (25) with *N*-bromosuccinimide gave the unsaturated bromo ester (26). The reaction of this crude product with potassium phthalimide gave ethyl 5-phthalimido-cyclohex-3-ene-1-carboxylate as a mixture of isomers from which the major product, the *cis*- isomer (27), was separated by crystallisation. Acid hydrolysis of (27) furnished the key intermediate (28) suitable for resolution. Resolution of (28) was accomplished by treatment with *L*- or *D*-ornithine, which formed crystalline salts which yielded the resolved phthalimido acids (29a) and (29b) respectively. Catalytic

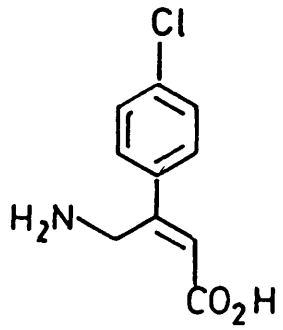


Reagents: i. NBS/CCl_4/AIBN; ii. Pht=N$^-K^+$; iii. Conc·HCl/H_2O; iv. Lor D-Ornithine; v. H_2/Pd/C; vi. CH_3NH_2/H_2O.

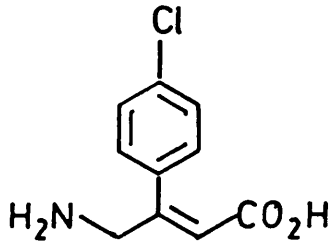
Scheme 4

reduction of these acids, followed by removal of the phthaloyl protecting group with methylamine, produced the two enantiomers (30a) and (30b) respectively. The (1S,3R) isomer showed a similar potency to GABA as an inhibitor of the uptake of radioactive GABA by rat brain slices, whereas the (1R,3S) isomer was at least twenty times less potent.

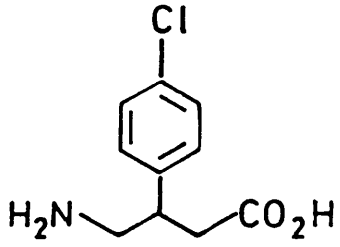
Both (Z)- and (E)-4-amino-3-(4-chlorophenyl)but-2-enoic acids (31a) and (31b)[32] have been synthesised from 4-chloroacetophenone as conformationally restricted analogues of the clinically useful drug baclofen (32) for further structure-activity studies. Thus, a Reformatsky reaction on 4-chloroacetophenone gave the α,β-unsaturated



(Z) (31a)



(E) (31b)



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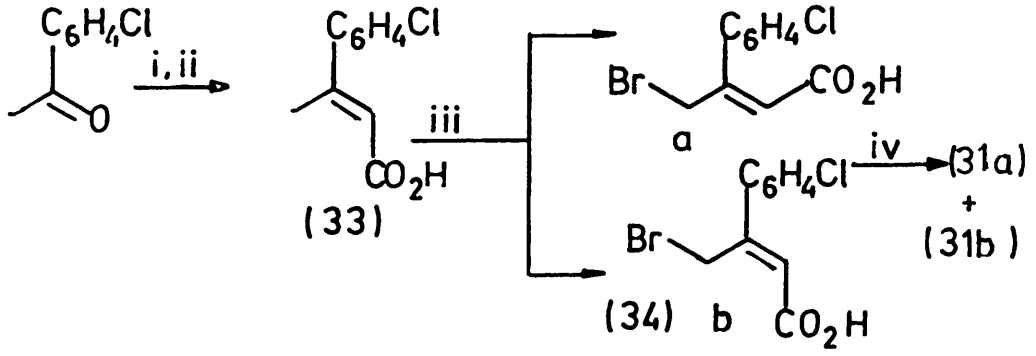
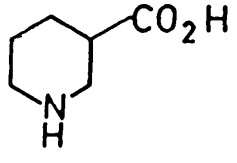


Reagents (i) $Zn/BrCH_2CO_2Et$, (ii) H^+/Δ, OH^-, (iii) NBS/CCl_4, (iv) Liq. NH_3 / THF.

Scheme 5

acid (33). Allylic bromination of this acid gave a 12:1 mixture of (Z) and (E)- isomers, (Z) being the major product (34a). Amination of these bromo acids with liquid ammonia gave the amino acids (31a) and (31b) respectively.

Nipecotic acid (35) is a selective and potent inhibitor of the neuronal uptake of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). For structure-activity studies, its derivatives, such as the *cis*-4-hydroxy and *cis*-5-hydroxy acids, as well as the α,β-unsaturated analogues guvacine (36) and others were synthesised. Results from these and other heterocyclic analogues indicated that a carbon-carbon double bond, or a hydroxyl functional group at the 4- position of (35) is consistent with activity as an uptake inhibitor. Allan *et al.*[33] synthesised the β,γ- unsaturated derivative of (35) with a double bond at the 4- position, especially compound (37). This compound was prepared from the commercially available methyl *N*-methyl-1,2,5,6-tetrahydropyridine-3-carboxylate (38) (Scheme 6). Demethylation of the free amine (38) was achieved by using 2,2,2-trichloroethyloxycarbonyl chloride and the resulting carbamate ester was deprotected with zinc-acetic acid. Acid hydrolysis yielded the bromide (39). Treatment of this salt with di-*t*-butyl dicarbonate and base



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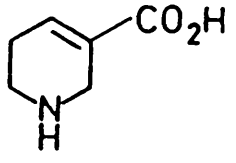
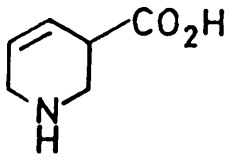


(36)



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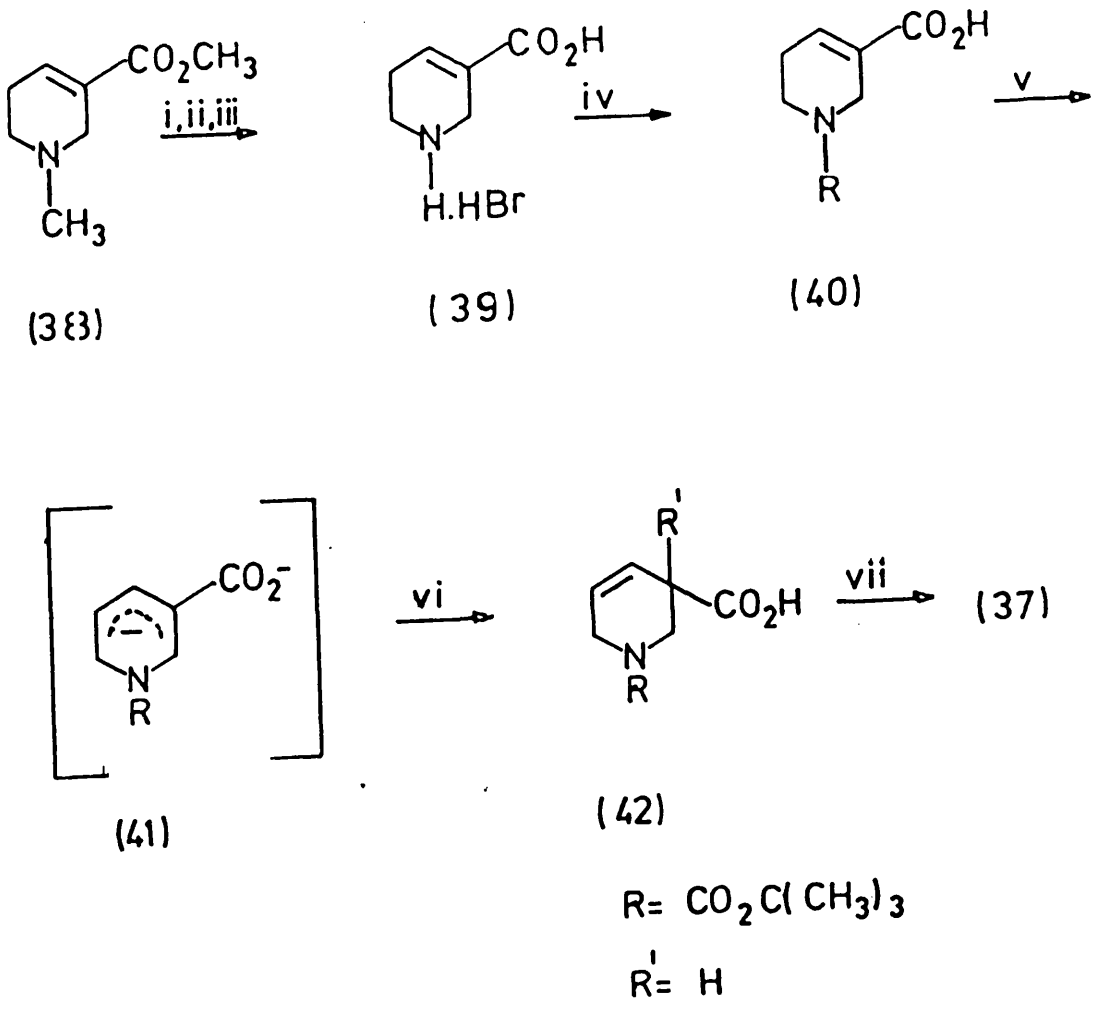
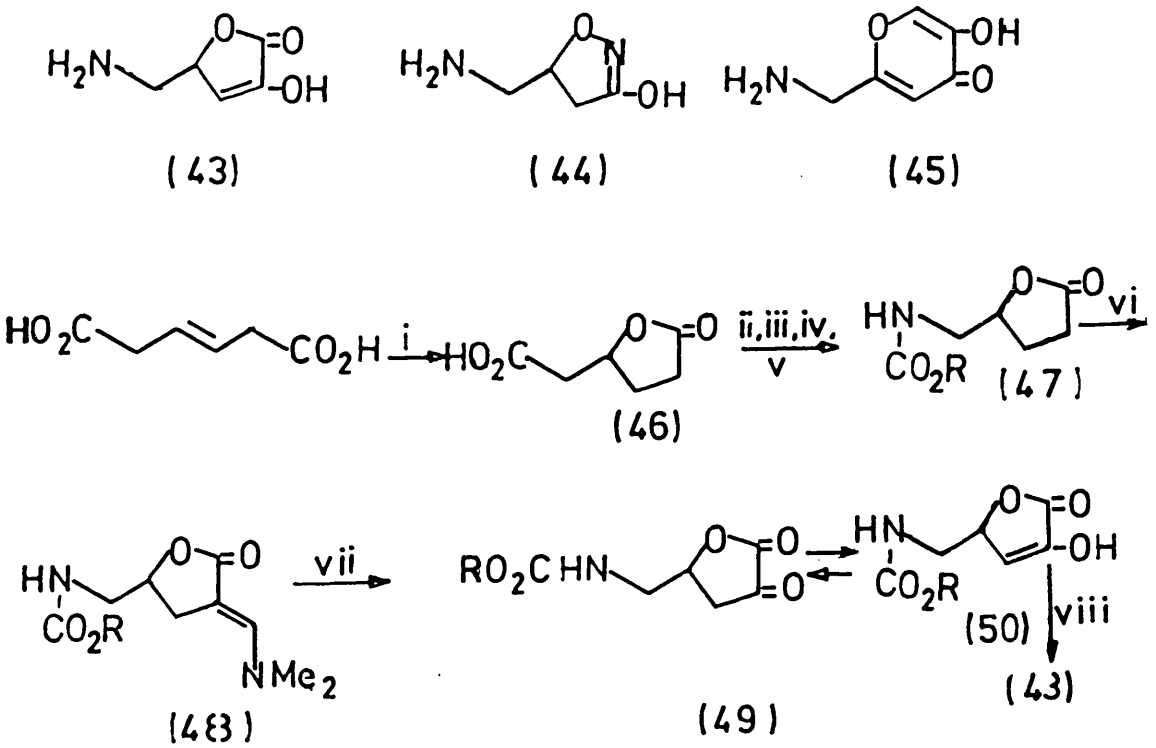


Reagents: i. Cl_3CCH_2OCOCl; ii. Zn/AcOH; iii. HBr; iv. $(Bu^tOCO)_2O$; v. LDA; vi. HCl; vii. HBr/AcOH.

Scheme 6

yielded compound (40). The dilithium enolate salt (41) gave (37) when quenched with hydrochloric acid, followed by treatment with HBr/AcOH.

The aminomethyl lactone (43)[34] was synthesised for further structure-activity studies because of its obvious structural relationship to GABA, dihydromuscimol (44) and Kojic amine (45), which are active at GABA receptor sites in the mammalian central nervous system.



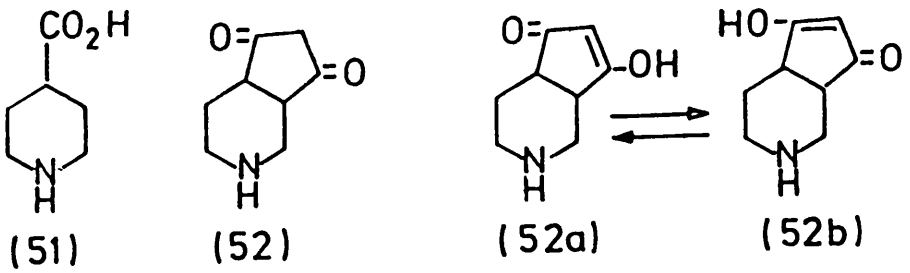
Reagents: i. $(C_{18}H_{17})_3N/\Delta$; ii. $SOCl_2$; iii. NaN_3; iv. Δ; v. ROH; vi. $Bu^tOCH(NCH_3)_2$; vii. $O_2/CH_2Cl_2/-78°C$; viii. HBr/AcOH

Scheme 7

The lactone (46) was available from (E)-hex-3-enedioic acid by heating with trioctylamine. This was converted into (47) by a Schmidt reaction sequence involving the acid chloride, acyl azide and subsequent addition of *t*-butyl alcohol or 4-methoxybenzyl alcohol. The reaction of the carbamate (47) with *t*-butyloxybis(dimethylamino)methane gave the enamine (48), which underwent dye-sensitised photo-oxygenation to the α-keto lactone (49). This compound isomerised to the enolic form. Removal of the protecting group with hydrobromic acid afforded (43).

This compound showed negligible activity as a GABA agonist with respect to inhibition of [^{3}H]-GABA binding, uptake and transamination in rat brain membranes.

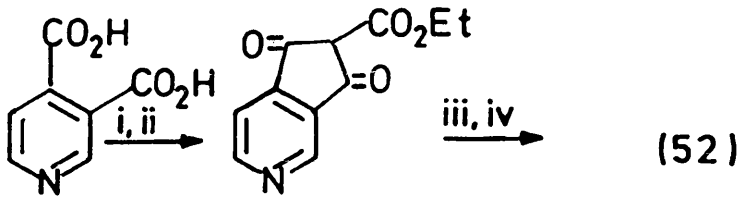
Compounds such as nipecotic acid (35) with an acidic functionality at the 3-position of a piperidine ring selectively inhibit the cellular uptake of the inhibitory neurotransmitter GABA. Others with an acidic functionality at the 4-position of the ring such as isonipecotic acid (51) act at postsynaptic GABA receptors with minimal interaction at GABA uptake sites. Since β-diketones, as their enolic tautomers, can be considered as vinylogues of carboxylic acids with comparable acidity,



(51) (52) (52a) (52b)

Allan *et al.*[35] synthesised compound (52), which may exist as tautomers

(52a) and (52b) and therefore capable of bearing an acidic functional group in positions corresponding to those of the acidic group in either (35) or (51). Thus the anhydride from cinchomeronic acid condensed

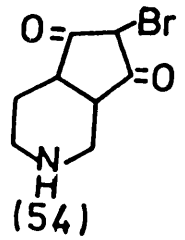


(53)

Reagents: i, Ac_2O., ii, $MeCOCH_2CO_2Et$/Et_3N, iii, H_2/Pt/NaOH., iv, HCl/Δ.

Scheme 8

with ethyl acetoacetate and cyclised under basic conditions to give the intermediate pyridine diketo ester (53). Selective reduction of the heterocyclic ring was achieved by protecting the diketone as the enolic sodium salt during catalytic hydrogenation to give (52) after acid hydrolysis and decarboxylation, (Scheme 8). The bromo- compound (54) and the diazo-derivative (55) were prepared under the standard bromination and diazo transfer conditions from (52), whose nitrogen was first protected with the *t*-butyloxycarbonyl group. Removal of the protecting groups was achieved by using hydrobromic acid/acetic acid and trifluoroacetic acid for compounds (55) and (54) respectively.



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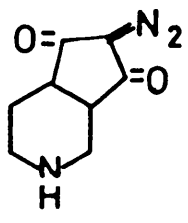
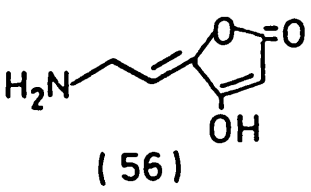


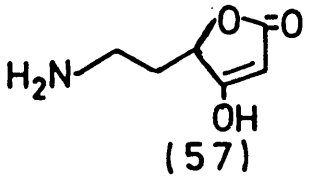
(55)

These compounds showed negligible or very weak activity as GABA agonists with respect to inhibition of [^{3}H]-GABA binding, uptake and transamination in rat brain membranes.

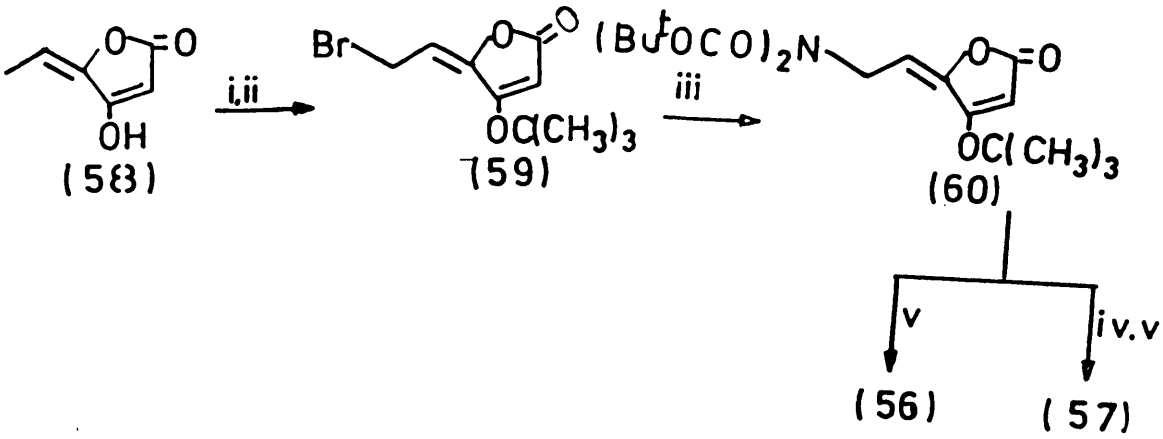
Tetronic acid derivatives were also investigated as potential GABA analogues by Allan *et al.*[36] and in particular, compound (56) was synthesised as a GABA analogue with the carbons of the GABA backbone constrained to be coplanar, as well as that of the considerably more flexible saturated derivative (57). Thus tetronic acid (58) was reacted with isobutene in the presence of sulphuric acid and brominated to give (59). The amino



(56)



(57)

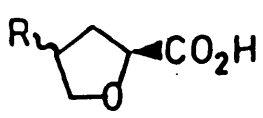


Reagents: i. $(CH_3)_2C=CH_2/H_2SO_4$.. ii. NBS.. iii. $(Bu^tOCO)_2N^-K^+$.. iv. $H_2/Pd/C$.. v. HBr/AcOH.

Scheme 9

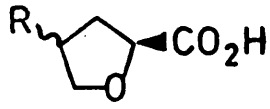
group was introduced by treatment of (59) with a potassium salt of bis(*t*-butoxycarbonyl)amine. Deprotection of (60) was achieved by treatment with hydrobromic acid in acetic acid (Scheme 9).

cis- And *trans*-4-aminotetrahydrofuran-2-carboxylic acids[37](61a) and (61b) were synthesised as conformationally restricted analogues of GABA from the *cis*- and *trans*-hydroxy acids (62a) and (62b). The hydroxy acids are



(61a) R = ◀ NH_2

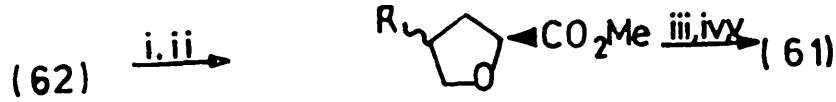
(61b) R = ||||| NH_2



(62a) R = ||||| OH

(62b) R = ◀ OH

ideal precursors for the amino acids and are available from diethyl allyl malonate by a literature procedure involving the separation of the stereoisomers by fractional crystallisation.[37] Thus the intermediate tosyl methyl esters (63a) and (63b) each underwent clean displacement reactions with sodium azide to yield, after catalytic reduction and hydrolysis, the



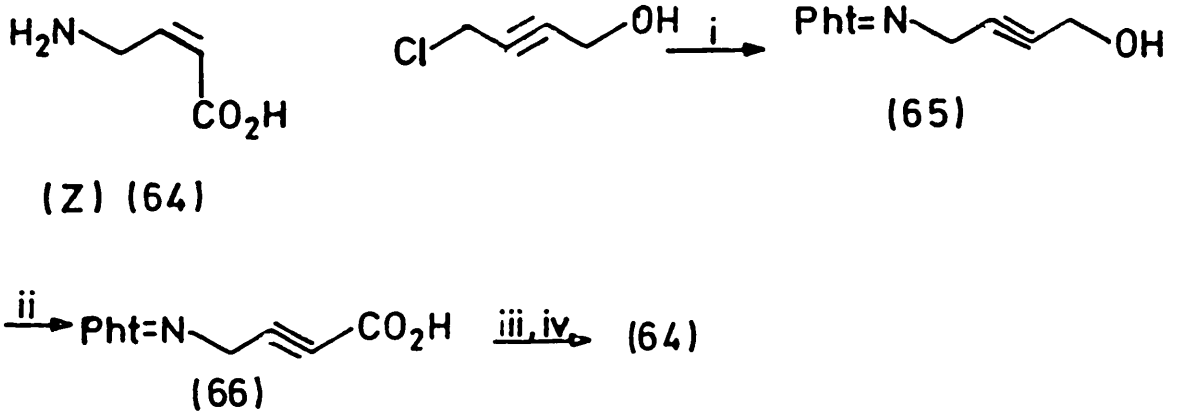
(62) —i,ii→ (63a) R = ||||| oTs; (63b) R = ◀ oTs —iii,iv,v→ (61)

Reagents: i. CH_2N_2., ii. TsCl., iii. NaN_3., iv. H_2 / Pd/C., v. H^+/H_2O

Scheme 10

required amino acids (61a) and (61b). The activity of these compounds, when compared with *trans*- and *cis*-cyclopentane analogues (8) and (9), was at least 100-fold less active at transiently contracting the isolated guinea-pig ileum, and were also not significantly active at inhibiting the uptake of radio labelled GABA into rat brain slices.

(Z)-4-aminocrotonic acid (64) and the more readily available (E) isomer was previously synthesised as conformationally restricted analogues of GABA[38] from 4-aminotetrolic acid which is difficult to purify. Since both isomers have different activity in conventional assays of GABA-mimetic activity, an alternative route to the (Z)-isomer free from the contamination of its (E)-isomer was sought by Allan *et al.*[40] after a recent finding that this isomer is a selective agonist for a new class of bicuculline–insensitive GABA receptor.[39] Allan's route utilised a crystalline phthalimide protected intermediate which could be purified and deprotected under mild conditions. Thus the phthaloyl-protected acetylenic amino alcohol (65), prepared from 4-chlorobut-2-yn-1-ol



Reagents: i. PhthN⁻K⁺., ii. CrO_3/ 0 °C., iii. H_2/Pd/C., iv. $EtNH_2$/EtOH

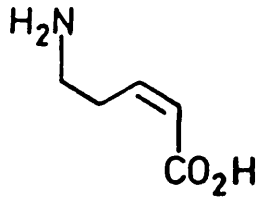
Scheme 11

was oxidised to the corresponding acid (66). Catalytic hydrogenation of (66) followed by removal of the phthaloyl protecting group afforded (64). Hydrogenation of (65) afforded the corresponding (Z)-alcohol whose oxidation with Jones reagent proceeded with complete isomerisation of the double bond.

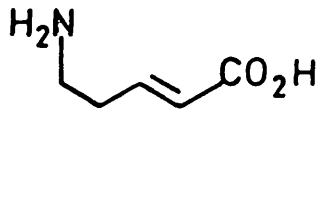
Johnston and Allan[41] have shown that the isothiouronium salt (67) is a very potent GABA-receptor agonist. Comparison of the charge separation of (67) with that of GABA and of 5-aminopentanoic acid suggested that a conformationally restricted δ-aminovaleric acid analogue may adopt a similar shape and charge separation at the receptor.

The compound which most closely resembles the potent GABA agonist (67) is the (Z) unsaturated amino acid (68). The corresponding (E)-isomer (69) can be considered as a homologue of (E)-4-aminocrotonic acid, a potent GABA-receptor agonist. For structure-activity studies on GABA, Allan *et al.*[42] synthesised compounds (68)-(72). Schmidt reaction on diacid (73) with 1 equivalent of sodium azide gave a good yield of the (E)-amino acid (71). Protection of the amino function was accomplished by treatment with di-*t*-butyl dicarbonate and subsequent methylation with diazomethane gave the *N*-*t*-butyloxycarbonyl ester (74). Conjugation of the double bond was readily effected by treatment with diazabicyclo[5.4.0]-undec-7-ene (DBU) to yield the (E) conjugated protected amino acid (75). The (E) amino acid (69) was obtained on deprotection of (75) with hydrochloric acid.

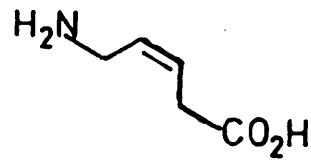
The (Z)-isomer (76) was generated by photolysis of the (E)-isomer (75) which gave a (Z)(E) mixture containing up to 35% of the required (Z)-isomer (76). Deprotection of this isomer yielded the (Z) amino acid (68). The diacid (79) was prepared from the known diol (78) by oxidation with Jones reagent. Treatment under Schmidt conditions with sodium azide and concentrated sulphuric acid gave the desired acetylenic amino acid (72). Partial reduction of the diol (78) in pyridine gave the (Z) olefinic diol (80), which was directly oxidised to the diacid (81) and converted into the amino acid (70) under the usual Schmidt conditions.



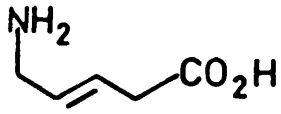
(Z) (68)



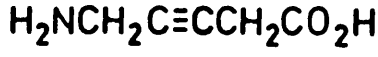
(E) (69)



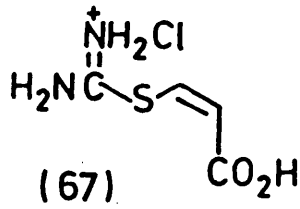
(70)



(71)

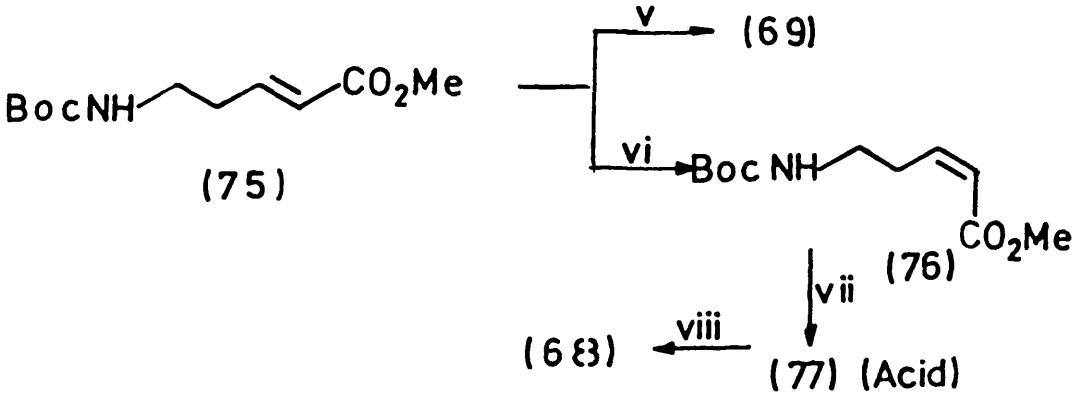


(72)



(67)

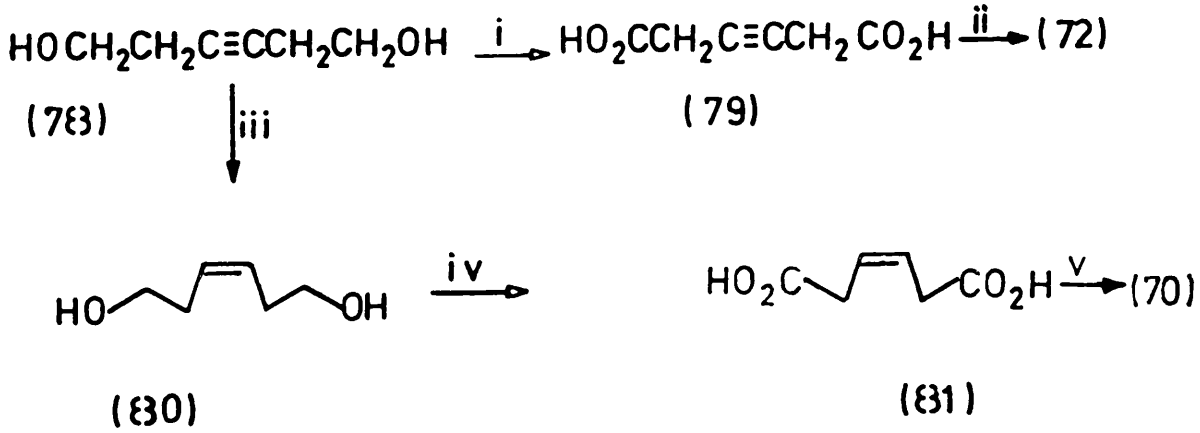
(E) (73) (74)



(75) (69) (76) (68) (77) (Acid)

Reagents: i. HN_3/H^+.. ii. $(Boc)_2O$ .. iii. CH_2N_2.. iv. DBU..
v. H^+/H_2O.. vi. $h\nu$.. vii. OH^-.. viii. H^+/H_2O.

Scheme 12

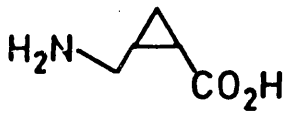


Reagents: i. CrO_3., ii. HN_3/H^+., iii. $H_2/Pd/C$., iv. CrO_3., v. HN_3/H^+.

Scheme 13

Only the (Z)-isomers were active as GABA agonists with (Z)-5-aminopent-2-enoic acid being two- to four-fold more active than 5-aminopentanoic acid.

Allan *et al.*[43] have also investigated the biological activity of two GABA analogues in which a cyclopropane ring holds the carbon atoms of the GABA chain in distinct rigid conformations, but without constraining them to be in a plane. They compared these cyclopropane derivatives with *cis*- and *trans*-4-aminocrotonic acid to ascertain whether substitution of non-planar methylated cyclopropane moiety for a planar olefinic moiety substantially influences the activity of these compounds as analogues of GABA. They therefore synthesised compounds (82) and (83). The reaction of acrylonitrile and ethyl diazoacetate gave *trans*- and *cis*-isomers (84) and (85) of cyano esters, which were separated by fractional distillation. Catalytic reduction of the *trans*-isomer (84) in acetic anhydride gave an amide ester which was hydrolysed with 6 M hydrochloric

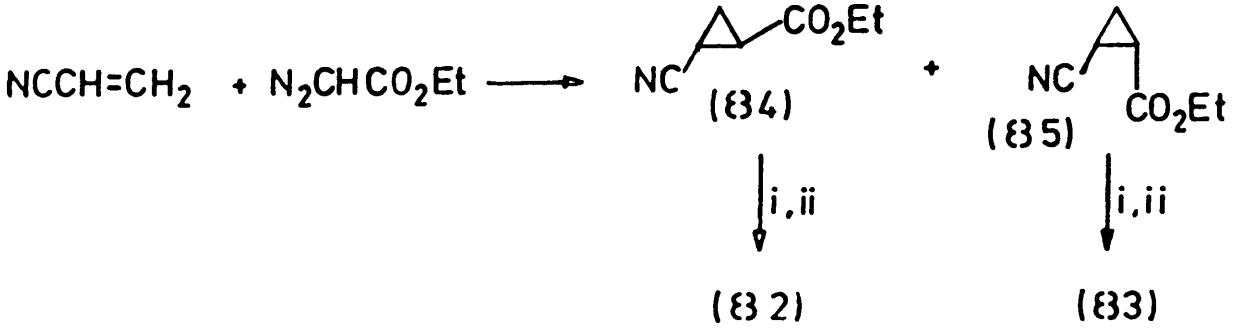


(Z)-CAMP

(83)

(E)- TAMP

(82)



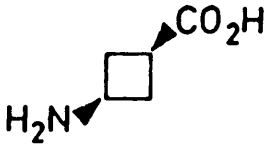
Reagents: i. H_2 /Adam's Catalyst., ii. 6M HCl.

Scheme 14

acid to afford *trans*-2(amino methyl)cyclopropanecarboxylic acid (TAMP) (82). Hydrogenation of the *cis*-isomer (85) under the same conditions was accompanied by considerable isomerisation with 50% of the *cis*- amide ester and 34% of the *trans*-isomer. The required *cis*-amino acid was obtained from this mixture after acid hydrolysis and careful recrystallisation.

Both isomers were found to be active as analogues of GABA[43] and reinforced the evidence that extended rather than folded conformations of GABA are active at most GABA recognition sites within the mammalian central nervous system.

Both *cis*- and *trans*-3-aminocyclobutane-1-carboxylic acids (86) and (87) were synthesised as conformationally restricted analogues of GABA by Allan *et al.*[44] In all the biological tests undertaken, the *cis*-isomer (86) was more active than the *trans*-isomer (87). This was interpreted in terms of the conformational pinning back of the polar groups



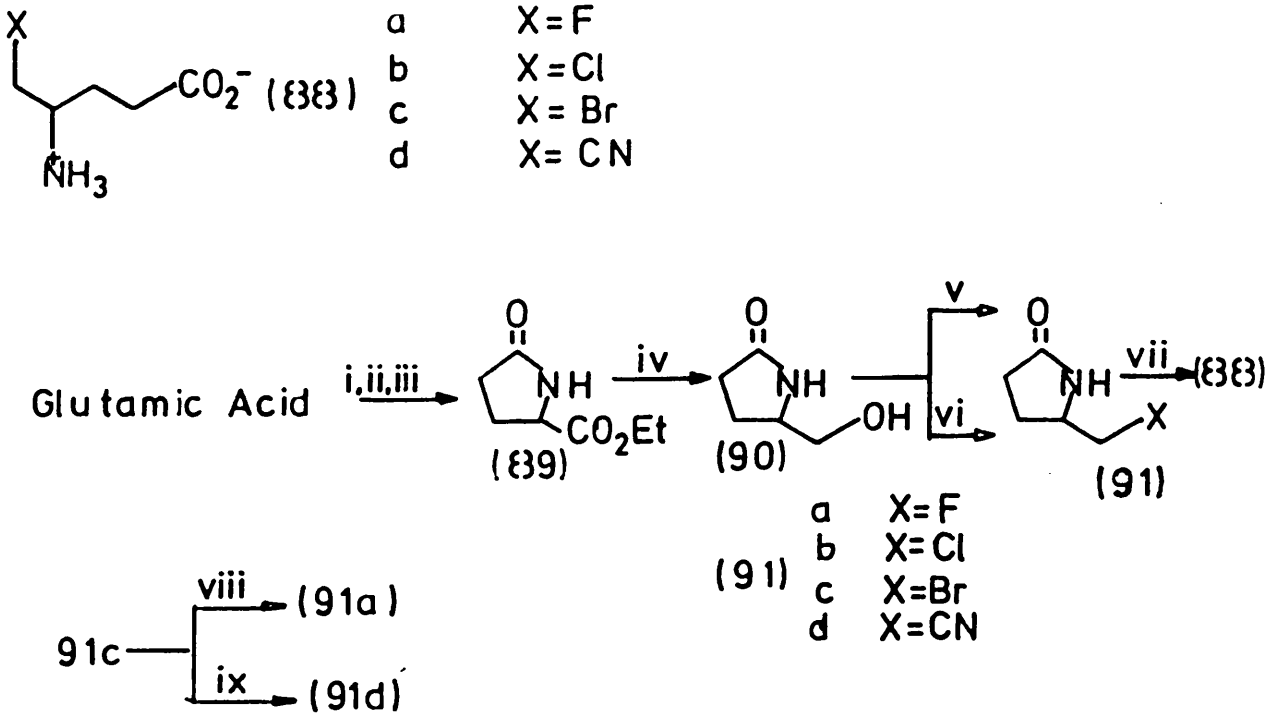
(86)

(87)

by the cyclobutane ring in (87) so that the unfavourable steric interactions would occur between one of the methylene groups and a region of steric hindrance at the active sites for particular GABA processes.

B R.B. Silverman and M. Levy

Silverman and Levy have synthesised (S)-5-substituted 4-aminopentanoic acids (88a)-(88d) as GABA-T inactivators.[45] The synthetic route involved the reduction of (S)-5-carbethoxy-2-pyrrolidinone derivative derived from glutamic acid to (S)-5-(hydroxymethyl)-2-pyrrolidinone (90). This alcohol was converted to the chloride or the bromide with triphenylphosphine and carbon tetrachloride or carbon tetrabromide, respectively. The fluoride (91a) was prepared from the bromide (91c) and silver fluoride, while the nitrile (91d), from the bromide and sodium cyanide impregnated



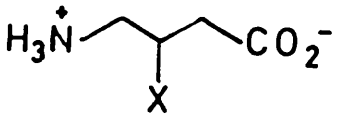
Reagents: i. $SOCl_2$/EtOH., ii. KOH. iii. 150 °C., iv. $LiBH_4$., v. CCl_4/PPh_3., vi. CBr_4/PPh_3., vii. HCl., viii. AgF., ix. NaCN.

Scheme 15

alumina. Each of the lactams was hydrolysed to the corresponding amino acid (88) with 1N hydrochloric acid without racemisation of the α-carbon of glutamate.

The 4-amino-5-halopentanoic acids (88a)-(88c) were found to be irreversible inactivators of pig brain GABA-T[46] and later evidence showed that they were mechanism-based inactivators.[47]

Substituted 4-aminobutanoic acids (92) were also studied as potential irreversible inactivators of purified pig brain GABA-T.[48] It was found

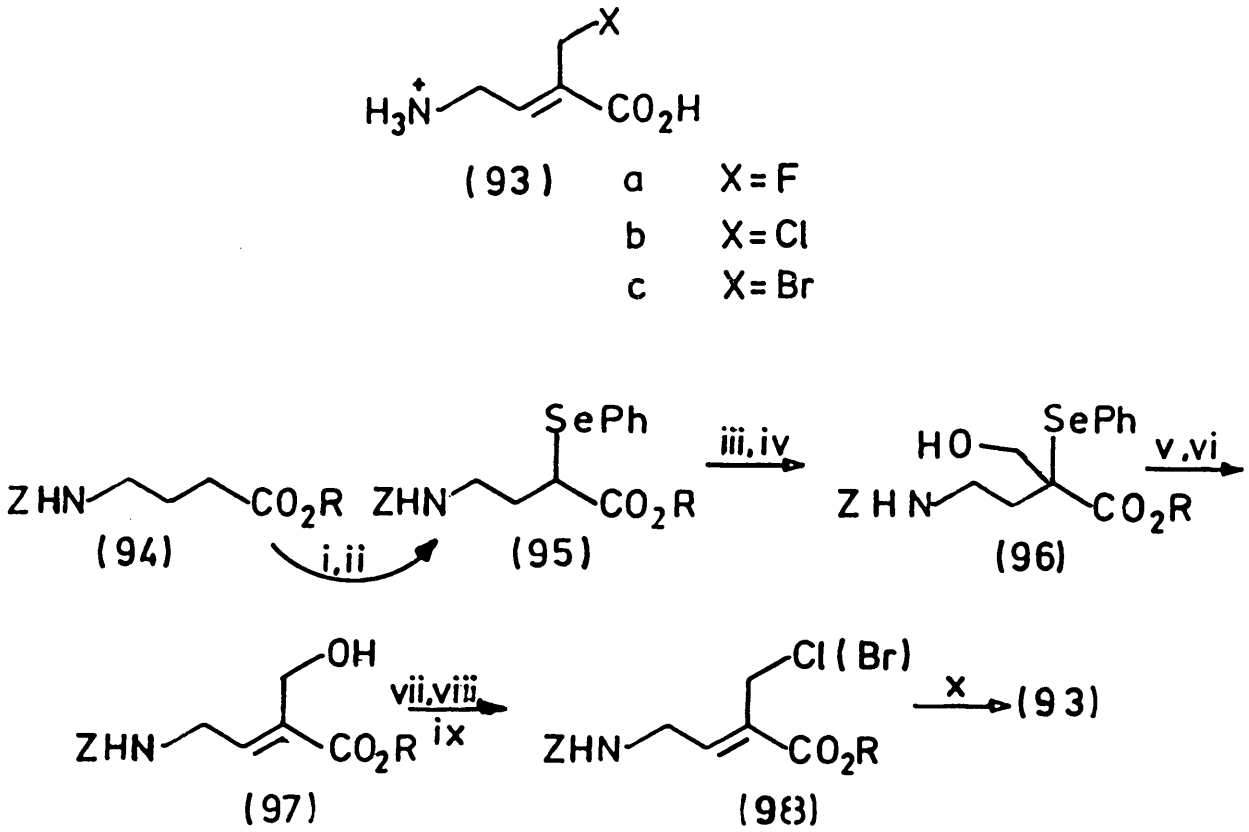


(R,S) (92)

a X = F
b X = Cl
c X = OH

that unlike the related 4-amino-5-halopentanoic acids (88), the 4-amino-3-halobutanoic acids were substrates for the enzymes undergoing exclusive elimination to succinic semialdehyde and producing no inactivation. The hydroxy analogue, however, underwent exclusive transamination and no succinic semialdehyde was detected.

Silverman *et al.* finally synthesised 4-amino-2-(substituted methyl)-2-butenoic acids (93)[49] and tested them for activity. The synthetic route involved the protected amino *t*-butyl ester (94). Thus, (94) was treated with LDA and phenylselenyl bromide to give (95). Further treatment of (95) with LDA and formaldehyde gave the alcohol (96). Elimination of the selenoxide was accomplished by treatment of (96) with *m*-chloroperbenzoic acid followed by heating to give compound (97). After activation of the alcohol in (97), displacement of the sulphonyl group with the chloride or bromide gave the chloride and bromide respectively. The fluoride was prepared by reacting the alcohol (97) with (diethylamino)-sulphur trifluoride in methylene chloride. Acid hydrolysis and deprotection gave (93).



Reagents: i. LDA / -78°C., ii. PhSeBr. iii. LDA/-78°C.,

iv. CH_2O., v. m-CPBA., vi. Δ., vii. TsCl,

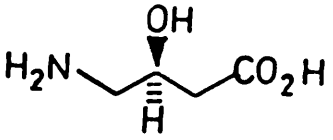
viii. LiCl (Br)., ix. Et_2NSF_3. For X= F.,

x. TFA / HCl.

Scheme 16

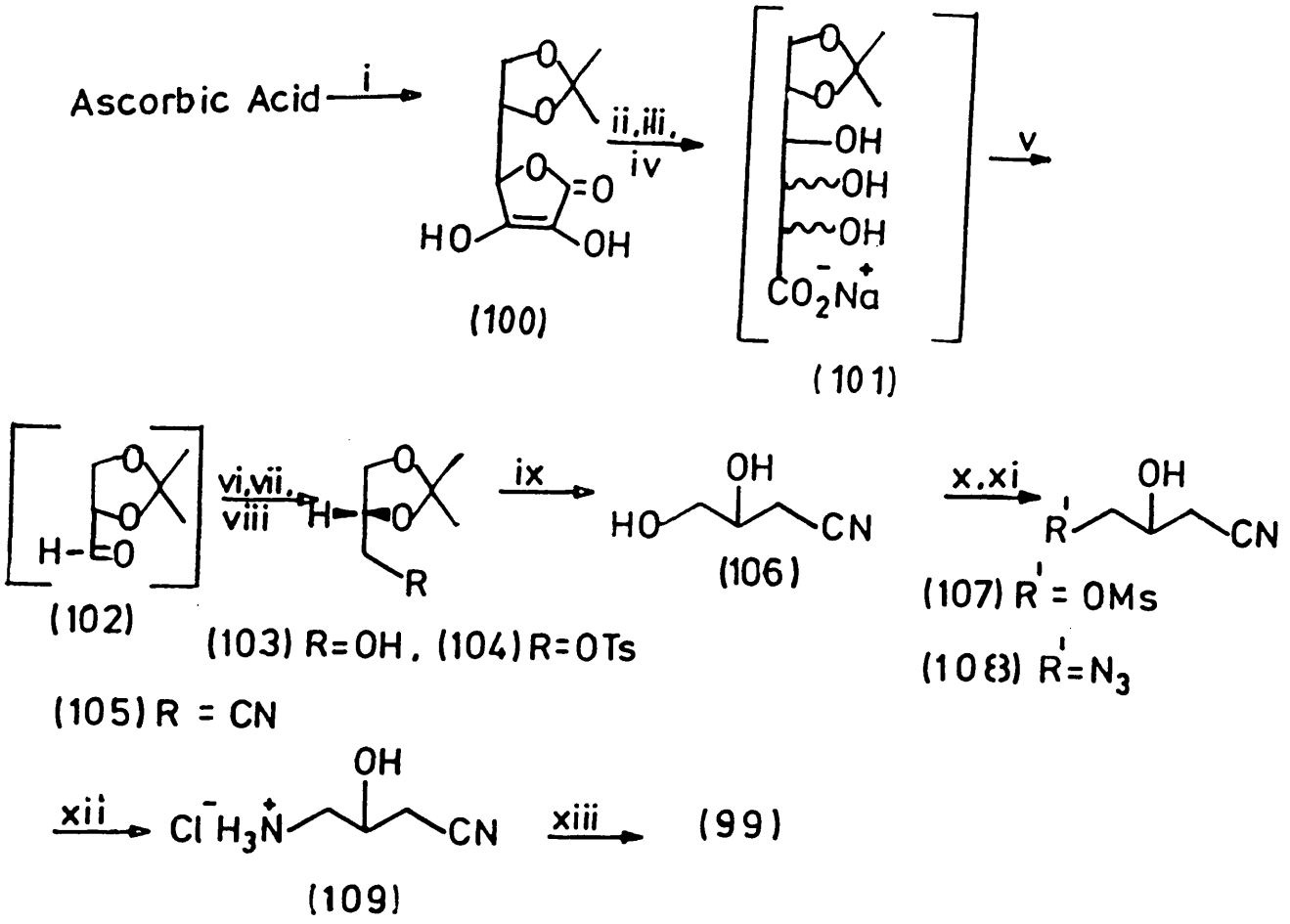
These compounds were found to be potent competitive reversible inhibitors. Unlike the previous two series of compounds, in which exclusive elimination occurs when the substituent is a halogen but exclusive transamination prevails for the hydroxyl-substituted analogues, in this series the fluoro- analogue gave a 4:1 ratio of elimination to transamination.[49]

Antiepileptic drugs, such as phenobarbitone and phenytoin have been shown[50] to increase the content of GABA in the brains of rats. This could contribute to their antiepileptic activity. However, they could also mimic or facilitate the action of GABA. Sodium valproate is an anticonvulsant which, *in vitro*, inhibits GABA-T. It could, therefore, impair the metabolism of GABA in the brain, leading to an increase in its concentration. This relationship between GABA levels and drugs with an anti-convulsant action[51,52] has led to the synthesis of the β-hydroxy derivative of GABA (92). The R-isomer of this racemic mixture is called GABOB (99) and is an antiepileptic and hypotensive drug. Up to



(99)

1979, all the known syntheses of GABOB involved a final resolution of the (±)-amino acid into the biologically-active (R)-(-)-enantiomer (99). The chiral synthesis of this enantiomer was established by Jung and Shaw,[53] using ascorbic acid (vitamin C) as a chiral starting material (Scheme 17). The saturated diol function of ascorbic acid was protected as acetonide (100) by treatment with excess acetone in the presence of a catalytic amount of acetyl chloride. Treatment of (100) with sodium

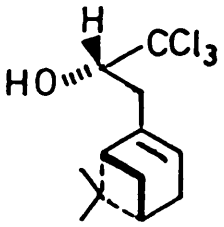


Reagents: i. $(CH_3)_2CO$/Cat. CH_3COCl., ii. $NaBH_4$., iii. NaOH., iv. H^+/ pH 7 ., v. $Pb(OAc)_4$ / EtOAc., vi. $NaBH_4$/NaOH., vii. TsCl/Et_3N., viii. KCN, NaI, $NaHCO_3$, $(CH_3)CO$., ix. HCl/MeOH, 0°C., x. MsCl/Et_3N., xi. KN_3/18-Crown-6/CH_3CN., xii. H_2/Pd-C, EtOH—$CHCl_3$., xiii. H_2SO_4/ H_2O.

Scheme 17

borohydride presumably reduced the ene-diol functionality. Cleavage of the borate esters and the lactone with excess hydroxide followed by exact neutralisation produced the acetonide carboxylate intermediate (101). This was further treated with 3.5 equivalents of lead tetra-acetate to cleave all the glycol bonds, and produce the unstable (S)-glyceraldehyde (102) in solution. This was immediately reduced with sodium borohydride to give

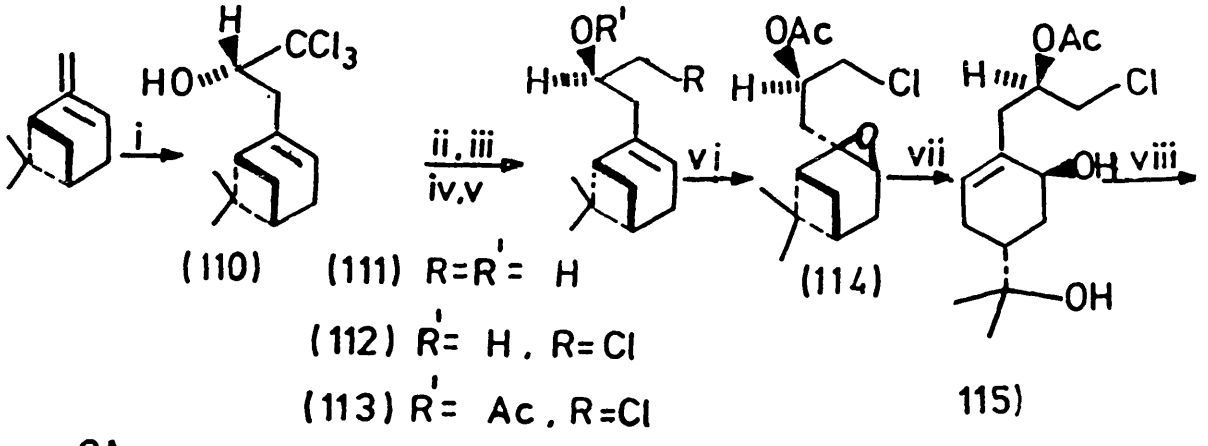
(R)-glycerol acetonide (103) after final work up with hydroxide. The alcohol (103) was converted into the nitrile (105) *via* the tosylate (104). Regeneration of the diol (106) and activation of the primary alcohol in (106) as a sulphonate ester allowed introduction of the nitrogen functionality by the displacement reaction with the azide ion. Catalytic hydrogenation of (108) gave (109) isolated as a hydrochloride salt. Acid hydrolysis of (109) afforded GABOB. This product was not, however, optically pure $[\alpha]_D$= -7.10, because optically pure forms of both S- and R-GABOB were later synthesised[54] from D- and L-arabinose respectively (S-GABOB$[\alpha]_D$ = 20.1, R-GABOB $[\alpha]_D$ = -20.7). Recently a stereocontrolled approach to R-(-)-GABOB has been established using (-)-β-pinene as a chiral promoter.[55] The study was carried out to clarify the contradictory evidence concerning the inversion or retention of stereochemistry at the exocyclic centre of (110) during alkaline hydrolysis of the trichloromethyl group. Thus (-)-β-pinene was reacted with

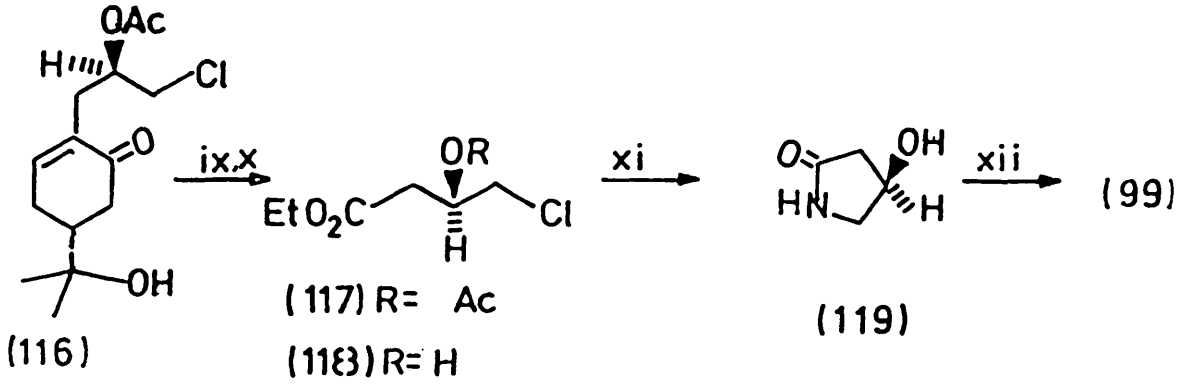


(110)

chloral catalysed by iron trichloride to give (110), possessing *S* configuration at the exocyclic centre. Alkaline hydrolysis of (110) gave the crude hydroxy acid with inversion at the exocyclic centre to (R) configuration. This crude product was further reduced to give the diol (111). The primary alcohol in (111) was converted into the chloride (112) by treatment with triphenylphosphine-carbon tetrachloride in pyridine. Acetylation of the secondary alcohol in (112) gave (113). Epoxidation of (113) and subsequent hydrolysis of the epoxide (114)

gave the unstable unsaturated alcohol (115), which was oxidised to the ketone (116). Further oxidation with RuO_2-$NaIO_4$ in a two-phase system gave, after esterification (oxalyl chloride and ethanol) (117), which was





Reagents: i. Cl_3CCHO / $FeCl_3$.., ii. NaOH/CH_2Cl_2/n-$Bu_4N^+HSO_4^-$.. iii. $LiAlH_4$/ THF.., iv. PPh_3-CCl_4/Py.., v. Ac_2O/DMAP/THF.., vi. MCPBA.., vii. 0·1N HCl/$(CH_3)_2CO$.., viii. Jones reagent /$(CH_3)_2CO$..,

ix. $RuO_2(H_2O)n$ $NaIO_4$ / CCl_4 / H_2O / CH_3CN;

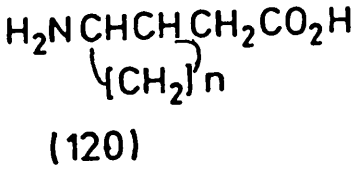
x. 3% HCl/EtOH.., xi. 25% NH_4OH.., xii. LiOH.

Scheme 18

deacetylated with ethanolic hydrochloric acid. Subsequent treatment with aqueous ammonia promoted intramolecular lactonisation to 4(R)-hydroxy-2-pyrrolidinone (119), which afforded GABOB after basic hydrolysis (Scheme 18).

C GABA analogues synthesised by other workers

Kennewell *et al.*[56] synthesised a series of the 2-aminocycloalkylacetic acids as GABA analogues of restricted conformation with restricted rotation about the C(3)-C(4) atoms. Compounds (120) were synthesised and tested for activity. In biochemical receptor binding studies, only the cyclopropyl and cyclobutyl amino acids showed any



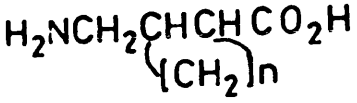
(120)

	n	isomer
a	1	*cis*
b	1	*trans*
c	2	*cis*
d	2	*trans*
f	3	*cis*
g	3	*trans*
k	4	*cis*
h	4	*trans*

significant biological activity. The *cis*-isomers (120a) and (120c) were found to be relatively weak inhibitors of [^{3}H]-muscimol binding (IC_{51} 100 and 49 μM respectively) to whole rat brain synaptic membranes. The *trans*-isomers (120b) and (120d) were found to be potent inhibitors of [^{3}H]-muscimol binding (IC_{50} 0.7 and 4.4 μM respectively). All the amino acids in this series were found to be inactive (IC_{50} > 100 μM) as inhibitors of [^{3}H]-GABA uptake.

They also synthesised the 2-(aminomethyl)cycloalkane carboxylic acids[57] (121) where free rotation about the C(2)-C(3) bond of GABA is

restricted by incorporating this bond into a cycloalkane ring. In receptor binding studies, only the *trans*-cyclopropane and cyclobutane derivatives showed any significant biological activity.



(121)

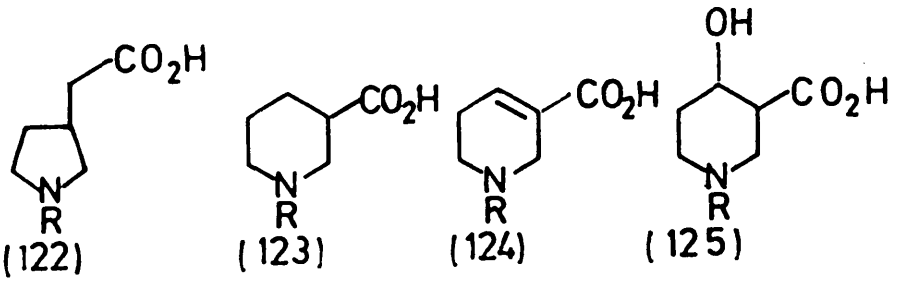
	n	isomer
a	1	*cis*
b	1	*trans*
c	2	*cis*
d	2	*trans*
e	4	*cis*
f	4	*trans*

All the amino acids in this series were found to be inactive as inhibitors of [^{3}H]-GABA uptake.

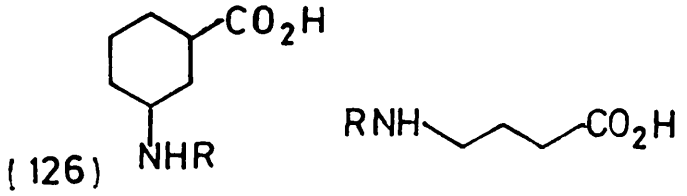
Shashoua *et al.*[58] synthesised labelled and unlabelled aliphatic and steroid esters of γ-amino[U-^{14}C]-butyric acid (GABA) directly from GABA by esterification with the corresponding alcohols. They tested these esters for their capacity to penetrate the blood-brain barrier and for evidence of central neuropharmacological activity in rodents. They found an effective GABA-delivery system that can produce apparent central GABA-like behaviourally depressant activity, possibly by sustained and slow release of GABA by local hydrolysis in the brain.

Galzigna *et al.*[59] have also demonstrated that GABA methyl ester hydrochloride is able to cross the blood-brain barrier after intracardiac administration to the rat. Once in the brain, it is hydrolysed to GABA by brain homogenates.

Ali *et al.*[60] have reported that appropriate *N*-alkylation of amino acids (122a-125a) yielded specific GABA-uptake inhibitors that are more potent, more lipophilic and, in limited testing, at least as selective as the parent amino acids. These parent amino acids do not readily



(122) (123) (124) (125)



(126) (127)

(a) R= H . (b) R =$(CH_2)_2CH=CPh_2$. (c) R = $(CH_2)_3CH\ Ph_2$

(d) R= $CH_2CH=CPh_2$ (e) R = $(CH_2)_3CH=CPh_2$

enter the central nervous system in pharmacologically significant amounts following pheripheral administration. They found that (123b) was a specific GABA-uptake inhibitor that was more potent, more lipophilic and in limited testing as selective as (123a). They found similar results with the *N*-alkylated derivatives (122b, 124b and 125b). By contrast the derivatives (126b and 127b) were not more potent than the parent amino acids and appeared to inhibit GABA uptake, at least in part, by a non-selective mechanism of action. The compounds (122b-125b) exhibited anticonvulsant activity in rodents following oral or intraperiteneal administration.

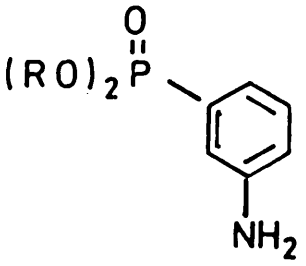
Other GABA analogues studied are the phosphorus analogues in which the carboxyl group is replaced by the $PO(OH)_2$ group. Bioulac *et al.*[61] investigated the activity of amino acids of structure (128). Several phosphoric analogues of excitatory and inhibitory ω-carboxylic acids were applied by microiontophoresis to rat cerebral and cerebellar neurones and showed that ω-phosphonic amino acids are able to interact with either

$H_2NCH(CH_2)n-PO_3H_2$
R_1
(128)

$R_1 = -CO_2H$. H. PO_3H_2
$n = 0, 1, 2$

excitatory or inhibitory post-synaptic receptors of ω-carboxylic amino acids. The application of 3-aminopropylphosphonic acid (GABA-P), for example, provoked inhibition in all the central neurones examined. The inhibition was somewhat stronger than that induced by GABA, and that unlike GABA, bicuculline methochloride or picrotoxine regularly failed to antagonise the inhibition induced by GABA-P. If the R_1 group was replaced by 'H', the compound was an inhibitor, whereas the presence of the acidic group in the vicinity of the amino group (R_1) was correlated with excitatory properties.

Cates *et al.*[62] synthesised a series of phosphorus compounds, designed as analogues of GABA in that they possess a P=O moiety separated by three atoms from an amino or acetamido group. They were tested by using *in vitro* $GABA_A$ and $GABA_B$ receptor binding, GABA uptake assays and were examined for anticonvulsant activity. Weak $GABA_B$ receptor affinity was noted for one agent, while other compounds displayed moderate to high potencies as inhibitors of electroshock- and pentylenetetrazol-induced seizures. The best anticonvulsant effect was found with the (*m*-aminophenyl)phosphonic acid compounds such as (129).



R = H, CH_3, C_6H_5, Et, $(CH_3)_2CH$.

(129)

1.2.2 Synthetic GABA Analogues Structurally Related to Muscimol

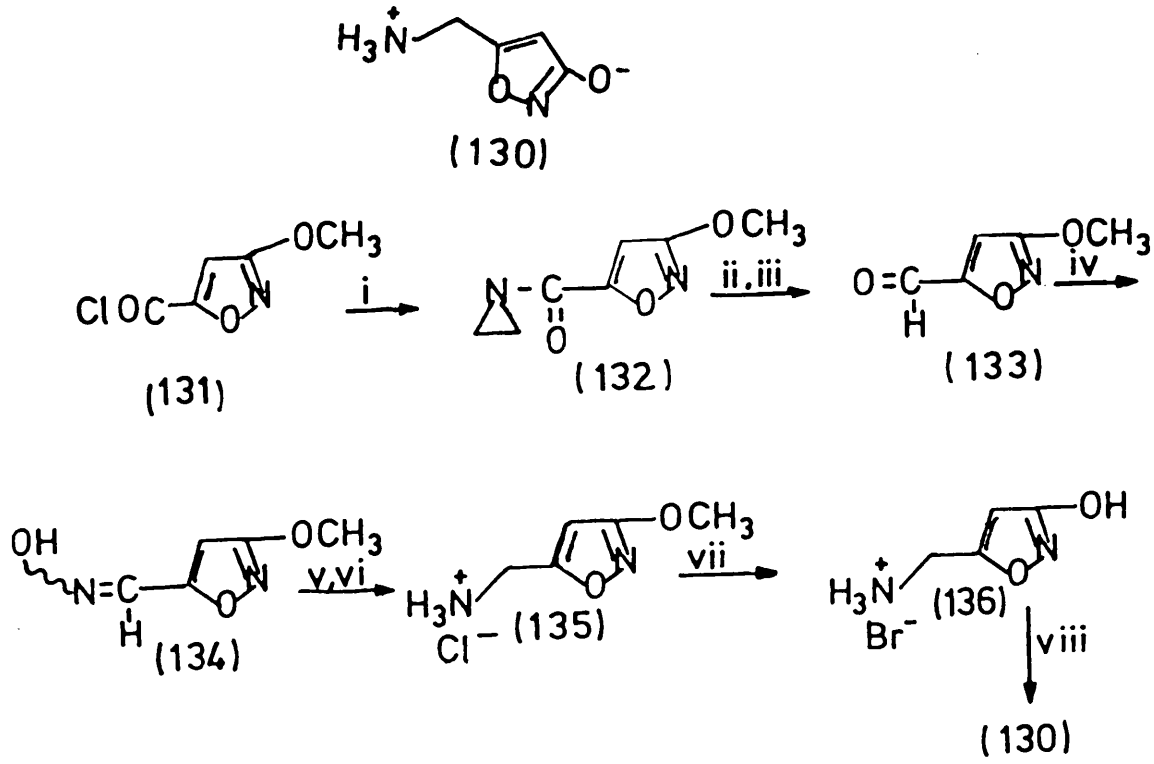
Another great contribution to the development of GABA analogues has been made by Krogsgaard-Larsen and his co-workers. They synthesised a series of compounds, some of which are structurally related to muscimol and tested them for activity.

P. Krogsgaard-Larsen and Co-workers

Muscimol (130), an isoxazole enol-betaine, was isolated from *Amanita muscaria*. Its isolation and elucidation of the structure has been described by four independent groups of workers.[63] Its synthesis has been reported by Gagneux *et al.*[64] *X*-ray analysis of muscimol has been performed by Krogsgaard-Larsen *et al.*[65] Muscimol has been demonstrated to be a GABA agonist of restricted conformation.[66] Its interference with the central inhibitory GABA neurotransmitter system, shows *in vitro* a very high affinity for postsynaptic GABA receptors and also for the presynaptic autoreceptors assumed to regulate the synaptic release of GABA. *In vivo* it is capable of activating the GABA receptors in a manner similar to that of GABA itself. In contrast to GABA, it can, in spite of its Zwitterionic structure, penetrate the blood-brain barrier.[67] Although it is not a substrate

for GABA-T *in vitro*, it is very rapidly decomposed *in vivo* probably by a transamination reaction involving the amino methyl side chain. It is also toxic. These multiple effects of muscimol at GABA synapses limit its utility as a pharmacological tool.

Various widely different routes for the synthesis of muscimol have been published.[68] Krogsgaard-Larsen and Christensen[68] have described a convenient synthesis of muscimol. Their route utilised the acid chloride (131). This acid chloride was reacted with aziridine in the presence of triethylamine to give the unstable acyl aziridine (132)



Reagents: i, Aziridine., ii, $LiAlH_4$., iii, H_2SO_4, iv, NH_2OH.,
v, Al-Hg., vi, HCl, vii, HBr/AcOH.,
viii, Amberlite IRA 400.

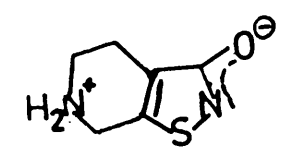
Scheme 19

which was treated with lithium aluminium hydride to give the aldehyde (133) after acid hydrolysis. Reaction of this aldehyde with hydroxylamine gave (134), which was reduced, with aluminium amalgam, to the amino compound (135) isolated as a hydrochloride. Dèmethylation of this compound with hydrobromic acid in acetic acid gave muscimol after treatment with a strongly basic ion exchange resin.

Krogsgaard-Larsen *et al.*[67] have written a review describing the syntheses of monocyclic muscimol analogues such as (RS)-4,5-dihydromuscimol,[69] thiomuscimol,[70] isomuscimol and 2-methylazamuscimol.[71] analogues of muscimol with different aminoalkyl chains[72-76] and bicyclic analogues[77,78] such as THIP, aza-THIP, thio-THIP and isoguvacine, in which the amino functions are incorporated into additional ring structures were all designed and developed as specific GABA agonists, while iso-THAZ[81] and iso-THIP[82] were found to have GABA antagonist properties.[83] Specific GABA uptake inhibitors such as THPO,[77] as well as guvacine and nipecotic acid and its analogues[79,80] were also developed.

The halides of thio-THIP (137), thio-THPO (138) and thio-THAZ (139) have been sythesised and tested for biological activity.[84] In contrast to THIP (140), thio-THIP (137) was a weak GABA agonist. Thio-THPO (138) was slightly weaker than THPO (141) as an inhibitor of GABA uptake *in vitro*, and these two compounds were approximately equipotent in enhancing the inhibition of the firing of cat spinal neurons by GABA. Like THAZ (142), thio-THAZ (139) was an antagonist at glycine receptors on cat spinal neurons.

The compounds (137)-(139) were synthesised from the appropriate β-oxo esters (143)-(145), which were converted into the β-oxo amides (146)-(148) with aqueous ammonia. These were converted into the



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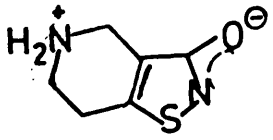

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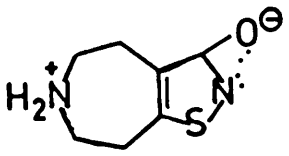

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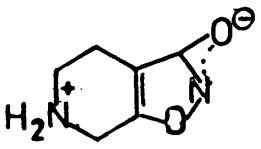

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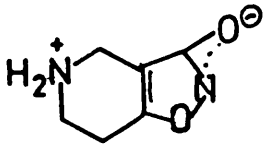

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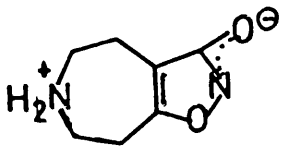
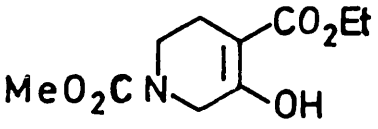


(142)

the stable β-enamino derivatives (149)-(151) by reaction with benzylamine. Treatment of these derivatives with hydrogen sulphide followed by oxidation of the intermediate thio compounds with bromine gave the 3-isothiazoles. Hydrolysis and decarboxylation of the carbamoyl functions with HBr gave products which were isolated as the hydrohalides (137)-(139).

N-substituted analogues of GABA, *trans*-4-methylaminocrotonic acid (152), *N*-methyl-muscimol (153) and *N*-methyl-thiomuscimol (154) were synthesised[85] and tested biologically and *in vitro*. They were found to be weak bicuculline methochloride (BMC)-sensitive GABA agonists.[85] Compound (152) was synthesised from ethyl-*trans*-4-bromobut-enoate



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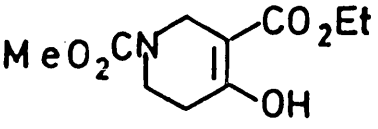

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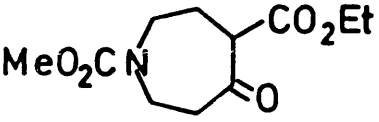

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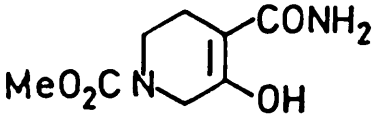

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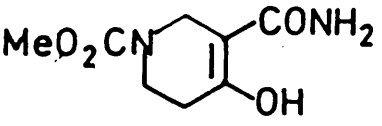

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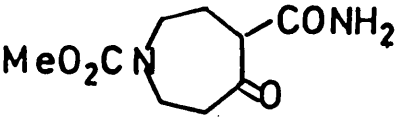

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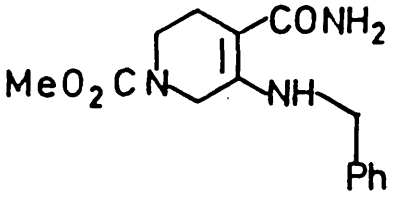

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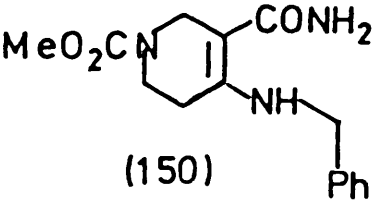

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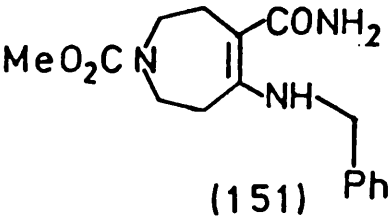
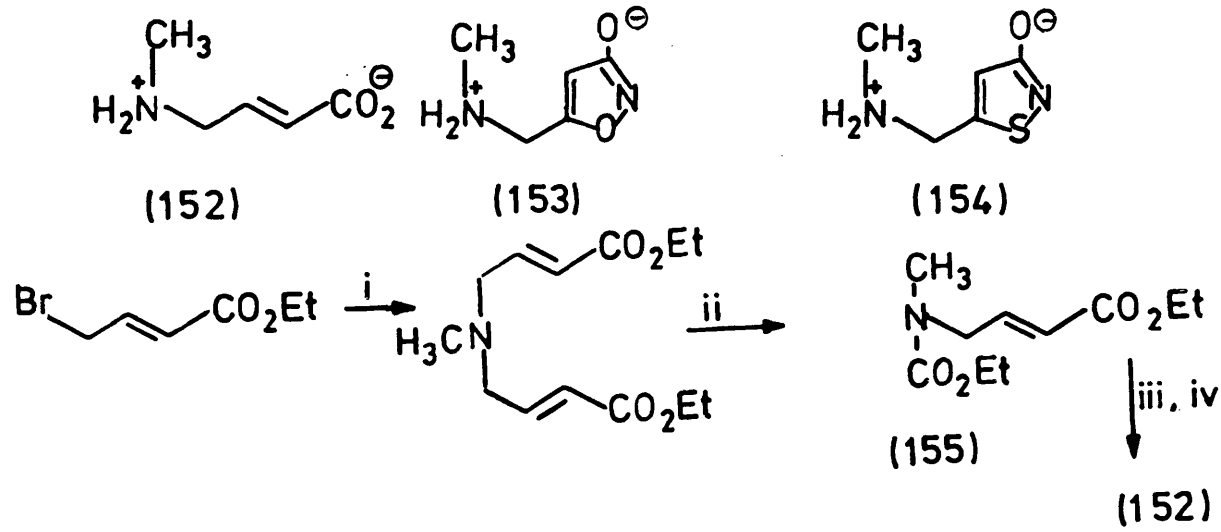


(151)

(Scheme 20). Treatment of this bromide with methylamine gave the tertiary amine, which on treatment with ethyl chloroformate gave the carbamate (155). Deprotection of this highly electrophilic intermediate was accomplished by using aqueous sulphuric acid followed by treatment with a strongly basic ion exchange resin.



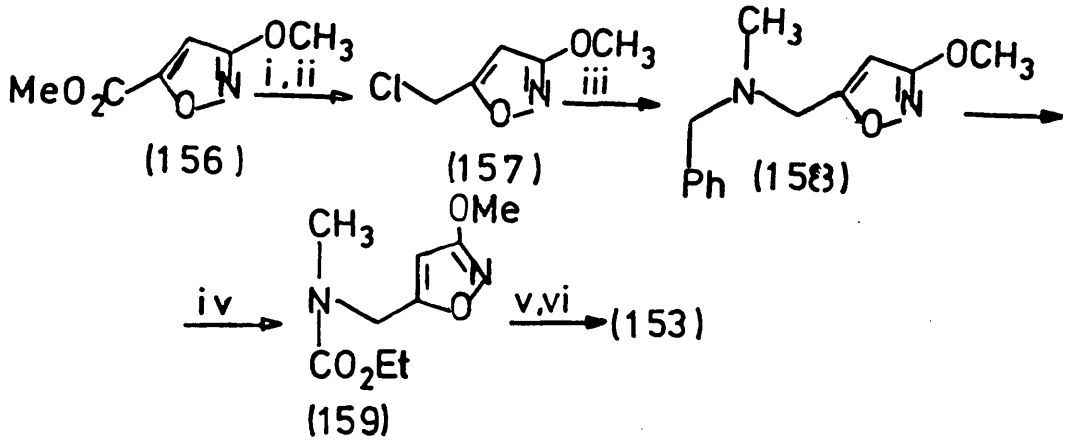
(152) (153) (154) (155)

Reagents: i. CH_3NH_2., ii. $ClCO_2Et$, iii. H_2SO_4.,

iv. Amberlite IRA-400.

Scheme 20

Compound (153) was synthesised using (156) as starting material (Scheme 21). Sodium borohydride reduction of (156) gave the alcohol



(156) (157) (158) (159)

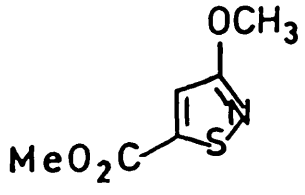
Reagents: i. $NaBH_4$., ii. $SOCl_2$., iii. $PhCH_2NHCH_3$., iv. $ClCO_2Et$.,

v. HBr/H_2O., vi. Amberlite IRA-400.

Scheme 21

which was converted into the tertiary amine (158) *via* (157).

Treatment of (158) with ethyl chloroformate gave the *N*-methyl carbamate (159). Demethylation, hydrolysis and decarboxylation was accomplished by using aqueous hydrobromic acid to give (153). Analogous reactions were used to convert (160) into *N*-methyl-thiomuscimol (154), although deprotection of the carbamate could only be accomplished by using hydrobromic acid in acetic acid. Finally, Krogsgaard-Larsen *et al.*[86] resolved (R,S)-5-(aminomethyl)-2-isoxazolin-3-ol(dihydromuscimol), a potent GABA agonist, the inhibitory effects of which on neurons are sensitive to the antagonist bicuculline methachloride (BMC). It also interacts with the GABA uptake system *in vitro*. (S)-(+)-DHM and

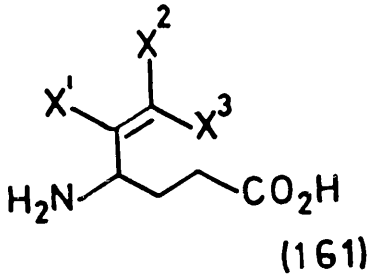


(160)

(R)-(-)-DHM were obtained in optically pure forms *via* resolution of *t*-butyloxycarbonyl-protected DHM using cinchonidine as the only resolving agent. It was found that the S-isomer was a specific and potent BMC-sensitive GABA agonist *in vivo* and *in vitro*, and the R-isomer was some fifty times less active than the S-isomer. The R-isomer was found to be an exclusive inhibitor of GABA uptake.

1.2.3 Recently Developed GABA-T Inhibitors and GABA-A Receptor Antagonists

A further addition to a series of GABA-T inhibitors was made by Kolb *et al.*[88] in a recent publication. They synthesised mono-, di-, and tri-fluoroethenyl-GABA derivatives, which are analogues of γ-vinyl-GABA (161a), a very selective enzyme-activated inhibitor of GABA-T in mammalian brain.[87] Their *in vitro* biochemistry showed that in the

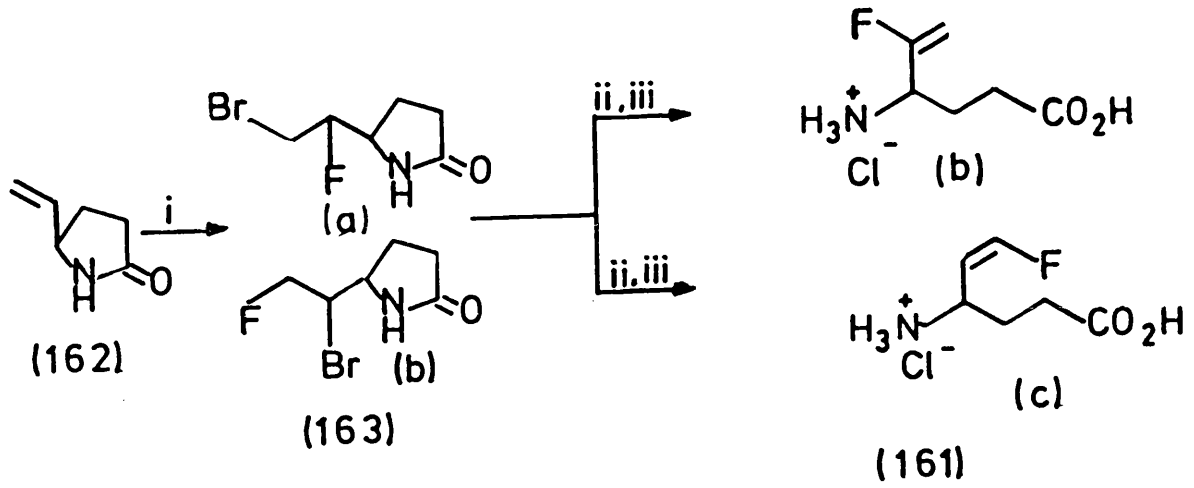


(161)

	X^1	X^2	X^3
a	H	H	H
b	F	H	H
c	H	H	F
d	H	F	F
e	F	F	F

series b-e, the least active inhibitor of GABA-T was the trifluoro-derivative (e), followed by the 'endo' mono-substituted compound (b). The 'exo' monofluoro- derivative (c) inhibited GABA-T at half the rate of γ-vinyl-GABA (a) and is the most active compound in the series. Compound (d) was as active as compound (a). *In vivo*, compound (e) was not active at all, while (b) was slightly active. The difluoro-derivative (d) was 42% active, but did not increase the level of GABA after inhibition of GABA-T. Again, the 'exo' derivative (c) was as potent as (a) at elevating brain GABA levels, despite a slightly lesser inhibition (38%) of GABA-T.

The compounds (161b-c) were synthesised from the 5-ethenyl-2-pyrrolidinone (162) (Scheme 22). Thus compound (162) was reacted with hydrogen fluoride in pyridine and NBS in ether to afford a 1:3 mixture of positional isomers (163a) and (163b). Treatment of this mixture with potassium *t*-butoxide in THF at -30 °C gave a mixture of fluoro- compounds after elimination of the elements of HBr. This mixture was separated by column chromatography. Hydrolysis, with hydrochloric acid, of each isomer, gave the desired compounds isolated as the hydrochlorides.



Reagents: i. HF/Py. NBS. Et_2O., ii. KO^tBu, THF, -78 °C → -20 °C.,

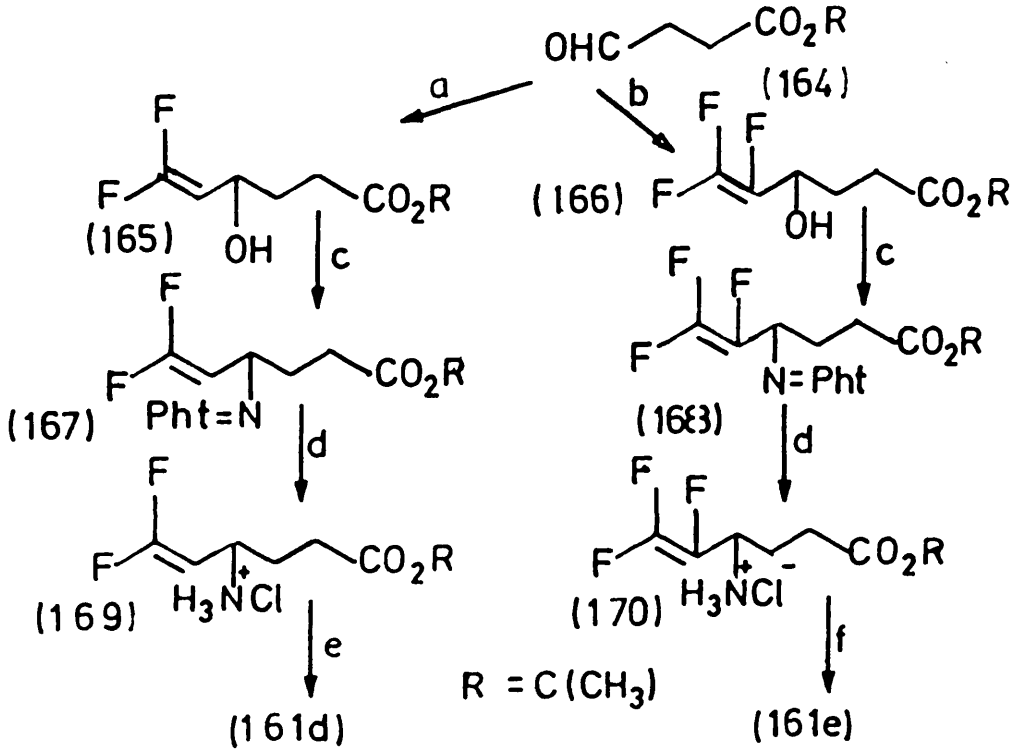
iii., 1N HCl, 30-35 °C, 15 h.

Scheme 22

Access to compounds (161d) and (161e) was gained *via* the aldehyde ester (164) (Scheme 23). Thus this aldehyde was reacted with (2,2-difluoroethenyl)lithium (from 1,1-difluoroethene) at -105 °C to give, after quenching at -50 °C, the fluorinated allyl alcohol (165). The amine functionality was introduced by reaction with phthalimide in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD) and the protected amine (167) was obtained. Hydrazinolysis followed by mild acid treatment gave the ester hydrochloride (169) which, on further acid hydrolysis, afforded the target molecule (161d).

Compound (161e) was analogously prepared from the aldehyde (164), with the exception that (trifluoroethenyl)lithium (from trifluoroethene)

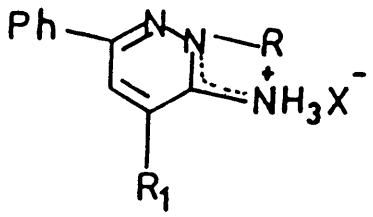
was used as a nucleophile in addition to the aldehyde (164) to give the trifluoroethenyl alcohol (166). Subsequent transformation to the protected amine derivative (168) and removal of the protecting group to obtain the amine ester chloride (170) afforded (161e) after final acid hydrolysis.



Reagents: a. $CH_2=CF_2$, sec-BuLi, -105 °C., b. $CF_2=CHF$, n-BuLi, -100 °C; c. PhthNH, PPh_3, DEAD, THF., d. H_2NNH_2, HCl., e. 1N HCl, 3 days, NaOH; f. 1N HCl, 3days, Et_3N.

Scheme 23

Wermuth *et al.*[89] have recently synthesised a series of 38 aminopyridazine derivatives of GABA (171) and (172) by attaching various pyridazinic structures to GABA or GABA-like side chains. These were tested and were found to be selective GABA-A antagonists. Most of the compounds displaced [^{3}H]-GABA from rat brain membranes.



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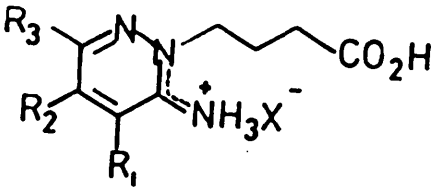


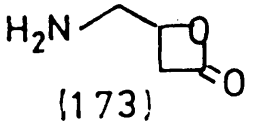
(172)

where R can be a carboxylic acid, ester, amide or a nitrile residue.

1.3 Proposed Target Molecules

The preceding introduction gave a brief account of the development and syntheses of various GABA analogues as either inhibitors of GABA-T, inhibitors of GABA-uptake, agonists or antagonists. Because GABA is a conformationally mobile neurotransmitter, we were concerned firstly with targeting and synthesising conformationally constrained variants which hitherto had not been described and which could be chiral. Secondly, we wished to prepare non-Zwitterionic species, since a major shortcoming of many Zwitterionic GABA analogues was their inability to penetrate the blood-brain barrier.

We therefore defined aminomethyl β-lactone (173) and aminomethyl γ-lactones (174) and (175) as synthetic targets, together with aziridinyl acetic acid (176) and aziridinyl propanoic acid (177).



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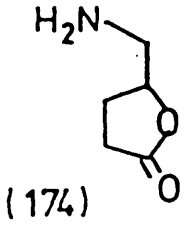

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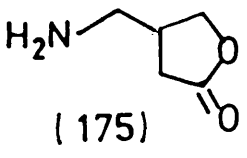

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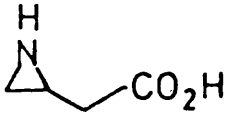

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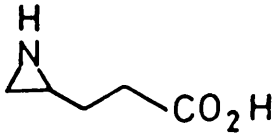


(177)

A further potential attraction of these systems was the fact that reduction of the number of degrees of freedom and resultant conformational restrictions would, if the molecule reached the GABA receptors and mimicked an important GABA conformation, result in entropic advantage accruing. Note also the possibility of exploring chiral requirements at the GABA receptor by synthesising chiral variants of the targets.

CHAPTER 2

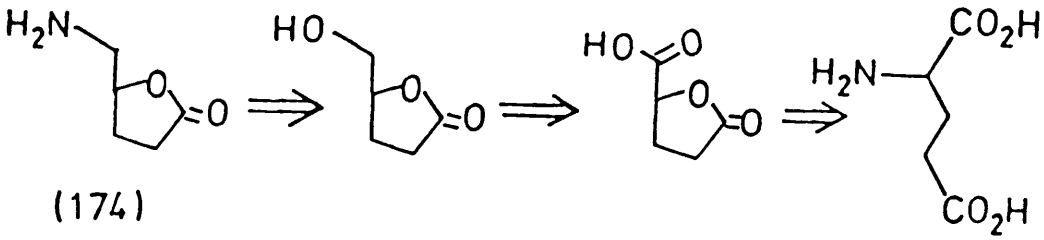
SYNTHESIS OF AMINOMETHYL-β- AND γ-LACTONES

This section describes the syntheses of the aminomethyl lactones (173), (174) and (175). As they all contain one asymmetric carbon atom in their skeletons, chiral synthesis was undertaken where possible. The synthesis of each lactone is described individually, since different strategies involved the construction of the lactone ring.

2.1 Chiral Synthesis of (R)- and (S)-(+)-γ-Aminomethyl-γ-butyrolactone

2.1.1 Introduction

The construction of the lactone (174) centred on the selection of an appropriate chiral starting material which would give the lactone with unambiguous stereochemistry at the asymmetric carbon. Retrosynthesis indicated that the hydroxymethyl lactone derived from glutamic acid was a potential chiral precursor (Scheme 24). Thus, both (R)- and (S)-isomers of glutamic acid (178) and (179) are potential useful chiral building blocks for the construction of the required lactone rings.

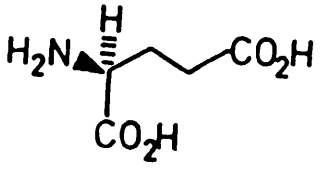


(174)

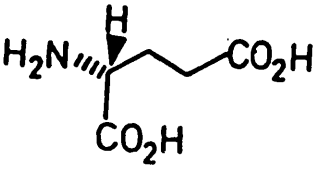
Scheme 24

It is known that the diazotisation and hydrolysis of α-amino acids proceed with retention of configuration[1b] due to the participation of the neighbouring carboxyl group through the formation of an intermediate α-lactone. Thus, when glutamic acid is treated with nitrous

acid, the diazonium cation interacts intramolecularly with the α-carboxyl

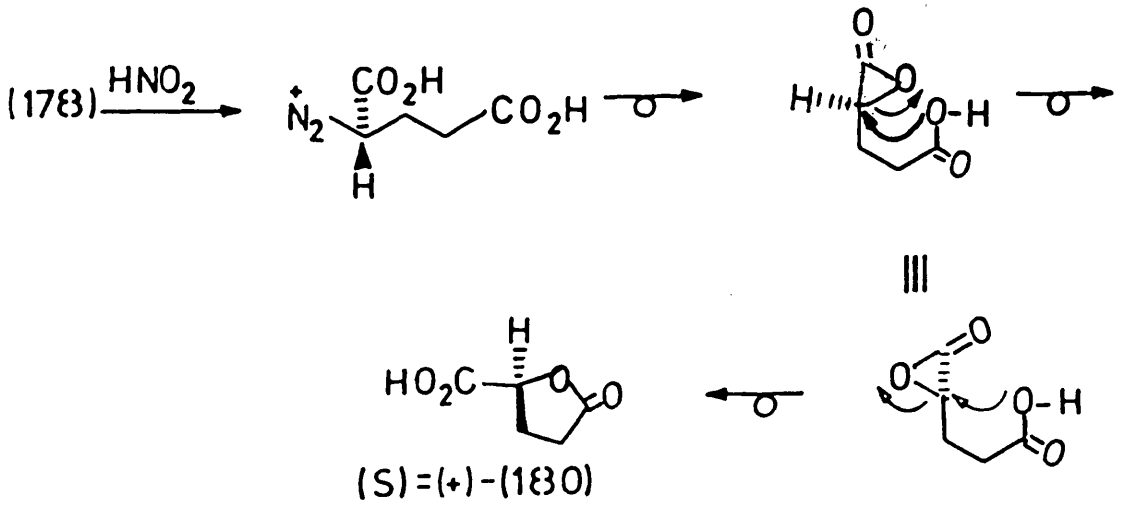


(S)-(+)-(178)



(R)-(-)-(179)

group to form a highly strained intermediate α-lactone with inversion of configuration. This reactive species is then rapidly ring-opened by the subsequent attack of the γ-carboxyl group, again with inversion of configuration, resulting in a full retention (Scheme 25).

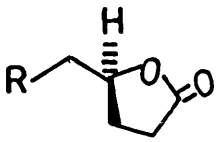


Scheme 25

2.1.2 Results and Discussion

S-Glutamic acid (178) was reacted with aqueous nitrous acid[1,4] at 0 °C to give the lactone (180) in quantitative yield. The crude product of this reaction was used directly in the next step. Selective reduction of the acid in the lactone (180) by borane-methyl sulphide,[4] furnished

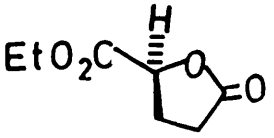
the (S)-(+)-γ-hydroxymethyl-γ-butyrolactone (181) in 75% yield (lit.,[4] 84%), whose IR and NMR spectra were consistent with the structure.



(181) R = OH

(183) R = OTs

(184) R = N_3



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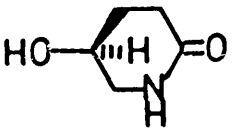
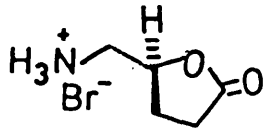


(185)

Alternatively, the acid lactone (180) was converted to the corresponding lactone ester (182) by reaction with ethanol, catalysed by *p*-toluene-sulphonic acid, in 59% yield (lit.,[4] 76%). Subsequent reduction of the ester with sodium borohydride[4] in ethanol at room temperature afforded the hydroxymethyl lactone (181) in 41% yield. The hydroxyl group of this lactone was activated by converting it to the 4-methyl-benzenesulphonate ester (183). Nucleophilic displacement of the sulphonate group in (183) with the azide ion was effected by refluxing with sodium azide in dimethylformamide. The azido lactone (184)[5] was obtained in 87% yield.

Catalytic hydrogenation of (184) with 10% palladium on carbon is known to furnish the lactam (185),[5] formed by the intramolecular attack of the amine on the carbonyl group, followed by the acyl-oxygen fission, thereby retaining the configuration of the asymmetric centre in the lactam formed. It is clear, therefore, that the amine would have to be in the form of a salt in order to prevent this undesirable reaction from taking place.

Hydrogen bromide[6,7] in acetic acid is known to reduce azides to amino compounds, as their hydrobromide salts. We therefore anticipated that by trapping the free amino group liberated during the hydrogenation of (184) as its hydrobromide salt, the lactamisation step could be prevented. So catalytic hydrogenation of (184) on 10% palladium on carbon in methanol in the presence of 48% aqueous hydrogen bromide at room temperature gave the crystalline (S)-(+)-γ-aminomethyl-γ-butyrolactone hydrobromide (186). The same compound was obtained when hydrogenation was conducted in the presence of chloroform, instead of hydrogen



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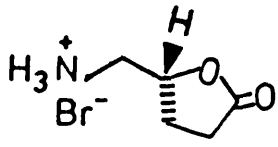

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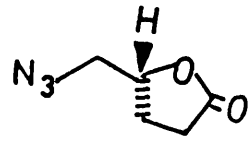


(188)

bromide and the aminomethyl lactone hydrochloride was isolated. Treatment of (184) with hydrobromic acid-acetic acid reagent, gave a complex mixture of products.

The IR spectrum of (186) had absorption bands at 1560 cm^{-1}, characteristic of the amino hydrohalide salts and 1750 cm^{-1} characteristic of the lactone carbonyl. The ^1H NMR spectrum recorded in D_2O was consistent with the structure. The α-methylene protons appeared at 2.40-2.80 ppm, while the β-protons appears at 1.82-2.24 ppm. The H_5 appeared as a multiplet at 3.0-3.44 ppm. The ^{13}C NMR spectrum was also consistent with the structure. The mass spectrum gave an expected ion at 115 (M-HBr). There was a 1:1 ratio of bromine isotope with mass units at 80 and 82, an indication of the presence of only one bromine atom in the molecule. The product also gave satisfactory elemental analysis. It was found to be optically active $[\alpha]^{23}$ 51.43, C, 1.55 (H_2O).

The same procedures were applicable to the synthesis of (R)-(-)-γ-aminomethyl-γ-butyrolactone hydrobromide (187). The unknown azide lactone (188) was prepared from the corresponding 4-methylbenzenesulphonyloxymethyl-γ-butyrolactone,[3] which was itself prepared from R-glutamic acid (179) by the same method as reported by Olsen *et al.*[5] and was isolated in 99% yield. The IR spectrum had absorption bands at 2100 cm^{-1} (azido) and 1770 cm^{-1} (C=O), The ^{1}H NMR and ^{13}C NMR spectra were identical to that of the S-isomer. The mass spectrum gave m/z 142 (M+1) corresponding to structure (188).

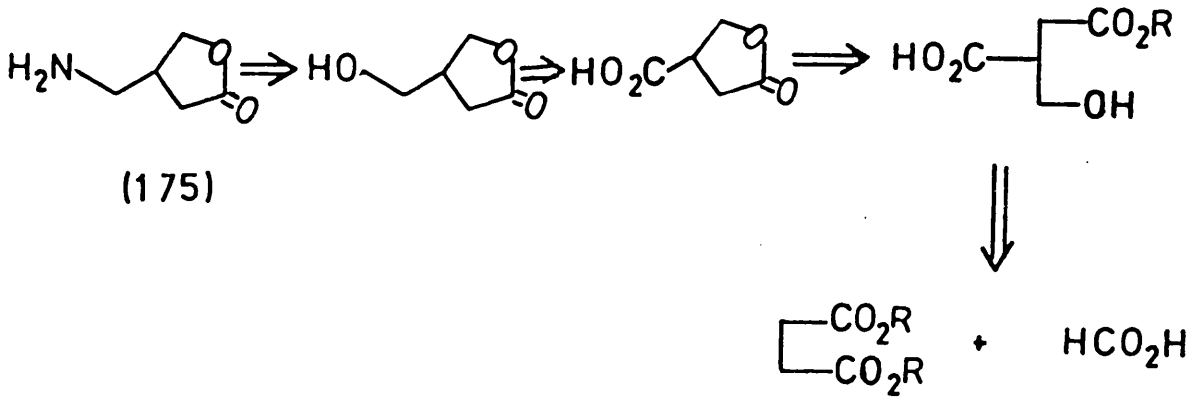
Catalytic hydrogenation of (188) in methanol at room temperature on 10% palladium on carbon in the presence of aqueous hydrogen bromide furnished the aminomethyl-lactone (187) in 86% yield. This also gave satisfactory spectral data. The IR spectrum (1560 and 1750 cm^{-1} respectively) and the ^{1}H and ^{13}C NMR spectra were consistent with the structure. It was found to be optically active with a value equal and opposite to that of the S-isomer. Satisfactory elemental analysis was obtained.

At the end of our work on this project, we became aware that closely similar work with related objectives had been carried out simultaneously by Herdeis.[8]

2.2 Synthesis of (R,S)-β-Aminomethyl-γ-butyrolactone

2.2.1 Introduction

The strategy used in the synthesis of this lactone was non-chiral, since no optically active precursors were readily available. Retrosynthetic analysis of the lactone (175) led to the simple and readily available starting materials shown (Scheme 26). The amino group can potentially be generated from the azido group by displacement of either



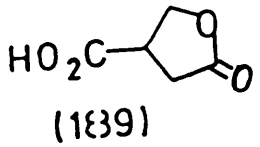
(175)

Scheme 26

the mesylate or tosylate. The alcohol is available from the lactone carboxylic acid which, in turn, is potentially available from succinic acid. The synthesis therefore centred on the preparation of this acid lactone.

2.2.2 Results and Discussion

(±)-Paraconic acid (189) has been prepared by Tocanne and Asselineau.[9]



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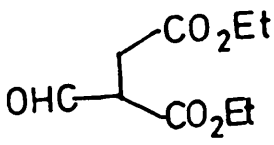

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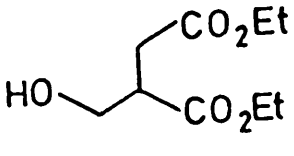
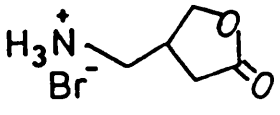


(191)

This became the starting point for the synthesis of our target molecule. The procedure involved the condensation of diethyl succinate with ethyl formate in the presence of sodium metal in ether at 0 °C. (±)-Diethyl formylsuccinate (190) was prepared in quantitative yield. Selective reduction of the aldehyde in (190) with sodium borohydride at 0 °C in just two hours furnished the alcohol (191) in 82% yield. Basic hydrolysis of the diester (191) with potassium hydroxide in aqueous

ethanol at refluxing temperature for one hour gave the salt of the diacid. Lactonisation was effected by treating this salt with an ion-exchange resin (Amberlite IR-120 in the H^+- form) to give (±)-paraconic acid (189) in quantitative yield. Reduction of this acid with borane-methyl sulphide[10] in THF at 0 °C gave the alcohol (192a) in 85% yield. Activation of this alcohol, as its 4-methylbenzenesulphonate ester (192b), was accomplished by stirring (192a) with 4-

(a) R = OH
(192) (b) R = OTs
(c) R = N_3



(175)

methylbenzene sulphonyl chloride in pyridine at 0 °C for 48 hours in the presence of a catalytic amount of *N,N*-dimethylaminopyridine, and was isolated in 74% yield, as a colourless homogeneous oil on standing. The ^1H NMR and ^{13}C NMR spectra were consistent with the structure. The lactone carbonyl still appeared at 1770 cm^{-1} as in the alcohol. High resolution mass spectrometry indicated a molecular ion at the expected mass value (m/z 270.0539).

The reaction of (192b) with sodium azide in refluxing *N,N*-dimethylformamide afforded the (±)-β-azidomethyl-γ-butyrolactone (192c) after one hour and was isolated in 77% yield after column chromatography on silica gel. The IR spectrum now had an absorption band at 2100 cm^{-1} characteristic of the azido group. ^1H and ^{13}C NMR spectra corresponded to the structure. The mass spectrum gave m/z 142 (M+1) required for structure (192c).

Catalytic hydrogenation of (192c) on 10% palladium on carbon in methanol at room temperature in the presence of 48% hydrogen bromide gave (±)-β-aminomethyl-γ-butyrolactone hydrobromide (175) as a colourless

wax both at room and lower temperatures. Crystallisation of this product was unsuccessful as the crystals melted just before isolation from the mother liquor. The IR spectrum showed absorptions bands at 1600 cm^{-1} ($\overset{+}{N}H_3$) and at 1760 cm^{-1} (C=O). The ^1H and ^{13}C NMR gave satisfactory spectra corresponding to structure (175). The mass spectrum gave the expected molecular ion at 115 (M-HBr). In view of the unsuccessfully crystallisation of this compound, it was not possible to obtain satisfactory elemental analysis.

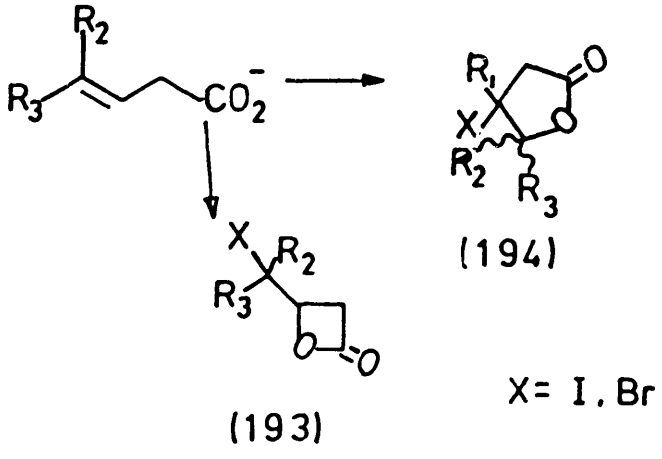
Thus (±)-β-aminomethyl-γ-butyrolactone (175) is readily available in good yield. No attempt was made to separate the enantiomers at the paraconic acid stage in order to prepare the target molecule in its chiral forms, because there was no evidence of biological activity in the GABA screens.

2.3 Attempted Preparation of (R,S)-β-Aminomethyloxetan-2-one

2.3.1 Introduction

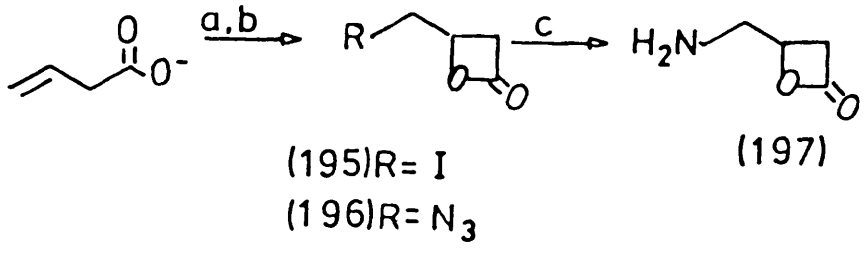
β-Lactones[11] are internal esters containing a strained four-membered heterocyclic ring. Consequently, they differ from γ- and δ-lactones both in general methods of their preparation and their chemical reactivity. They are usually prepared by: the reaction of β-halogen acids with basic reagents,[11,12] certain reactions of ketenes with carbonyl compounds[15] and the iodolactonisation[13] of β-γ-unsaturated acids.

We prepared this lactone by the third method.[13] β-Lactones (193) are the kinetic products in this process and they readily isomerise to the stable β-iodo-γ-lactones[16] (194). However, the rate of β-lactone formation is much faster than the rate of rearrangement to the γ-isomer, so that the β-lactone is easily detected as the kinetic product[14,17] (Scheme 27).



Scheme 27

The proposed synthesis of our aminomethyl-β-lactone centred on the preparation of the β-lactone[14] (193) from 3-butenoic acid. The β-azidomethyl-β-lactone (196) should be available from the iodolactone (195). Careful reduction of the azidomethyl lactone was planned to give the β-aminomethyl-β-lactone (197) (Scheme 28).



Reagents: a. I_2/Et_2O, 0°C., b. NaN_3., c. Reduction.

Scheme 28

2.3.2 Results and Discussion

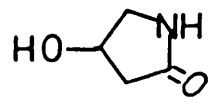
As explained in the introduction, the lactone (195) was prepared by a two phase technique.[14] This involved the conversion of 3-butenoic acid to the salt by dissolving it in a saturated solution of sodium bicarbonate to produce a homogeneous solution of the carboxylate salt. This solution was then added to iodine in ether at 0 °C. The β-lactone was extracted into ether as soon as it was formed, in order to prevent an equilibrium being set up which would yield the thermodynamically more stable γ-lactone. This is a 4-exo-Tet process which is favoured over the 5-endo-Tet process leading to the γ-lactone as predicted by Baldwin's rules[18] for ring closures. The yield of the product was very low (5%). This crude product was purified by column chromatography on silica gel and could be kept in the dark in an inert atmosphere at low temperatures for several weeks. The IR and NMR spectra corresponded to the structure and were consistent with those reported for the same compound.[14] A trace amount of the γ-lactone was detected in one of the fractions from the column, and in the crude product by the IR spectrum (1770 cm^{-1}).

In order to improve the yield of the β-lactone (195), 3-butenoic acid was reacted with iodine isocyanate, which was generated *in situ* from silver isocyanate and iodine in acetonitrile at 0 °C, for 6 hours and the lactone was isolated in 55% yield. There was still a significant amount of unreacted starting material, even after 6 hours. Employing a longer reaction period resulted in the yield being lower, due to the formation of the γ-lactone.

With the iodomethyl-β-lactone (195) in hand, the next task was the conversion of this unstable product into the azidomethyl-β-lactone (196). Scowen[19] first synthesised this compound in our laboratories

from the iodolactone (195). He reacted (195) with sodium azide in dimethylsulphoxide in the presence of silver tetrafluoroborate at room temperature. Silver tetrafluoroborate is a Lewis acid which complexes with iodine, thereby weakening the iodine-carbon bond, resulting in a facile SN2 displacement of the iodine by the azide ion. The yield was very low. We therefore repeated this procedure with a view to optimising the yield of (196). An extensive range of reaction conditions was investigated. For example, NaN_3-DMF gave no lactone even when the reagents were taken in excess and employing longer reaction time. However, the optimum conditions which eventually transpired involved use of excess reagents in DMSO for 7 days, and the required product was still isolated in less than 30% yield. The IR spectrum of this product included absorptions at 2100 cm^{-1} (azido) and 1110 cm^{-1} (C-O). The mass spectrum gave the expected ion at 127.

The next step was the reduction of the azido group in (196) to the target aminomethyl-β-lactone (197). The amine would probably need to be in the form of a salt in order to prevent intramolecular attack on the carbonyl group, resulting in the formation of a lactam (198). Also, β-lactone rings are readily opened by hydrogenation to form acids.[12] The solvent would also need to be dry as hydrolysis is one of the anticipated



(198)

reactions. Even alcohols hydrolyse[11] β-lactones under neutral, basic and acidic conditions to yield β-alkoxy acids, β-hydroxy acids and acrylic esters resulting from the dehydration reaction.

The azidolactone (197) was reduced by hydrogenation at room temperature on 10% palladium on carbon in dry methanol in the presence of 48% hydrogen bromide. After three days, IR analysis of the mixture indicated that the azido functionality had disappeared. The lactone ring had also opened, since no absorption at 1830 cm^{-1} was detected, but a carbonyl absorption at 1730 cm^{-1} was prominent. TLC analysis of the mixture indicated a complex mixture of products. The lactone ring had been opened, either by catalytic hydrogenation or by the action of hydrogen bromide. When ethanol was used as a solvent, the same result was obtained as in the case of methanol.

The reaction of (196) with hydrogen halides was next investigated. The reactions were conducted in dry dioxane or THF. The azidolactone (196) was first stirred in aqueous dioxane at room temperature overnight. Analysis by IR spectroscopy showed that the lactone ring was unaffected and was recovered. Dry hydrogen chloride was bubbled through a solution of (196) in dry dioxane. Within a few minutes the lactone ring disappeared, but the azido group was not affected. With hydrogen bromide, both the lactone and the azido group were affected. The IR spectrum of the mixture had no absorption bands at 2100 cm^{-1} and at 1830 cm^{-1}, but had bands at 3300-3500 cm^{-1} and at 1720 cm^{-1}, an indication that although the azido group was converted to the amino group, the lactone ring was hydrolysed in the same process. So it appeared that under standard conditions employed in the reduction of azido groups, the β-lactone ring undergoes ring-opening reactions, making it impossible to prepare the desired β-aminomethyl-β-lactone. Because of other synthetic priorities, no further efforts were made to synthesise this compound.

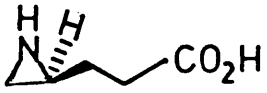
CHAPTER 3

APPROACHES TO THE AZIRIDINE-2-CARBOXYLIC ACIDS

3.1 Enantiospecific synthesis of (R)- and (S)-(+)-(2-Aziridinyl)-2-propanoic Acids

3.1.1 Introduction

This section describes the synthesis of (2-aziridinyl)-2-propanoic acids (199) and (200). The azidomethyl lactones (184)



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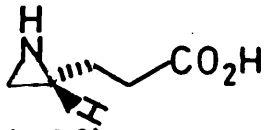
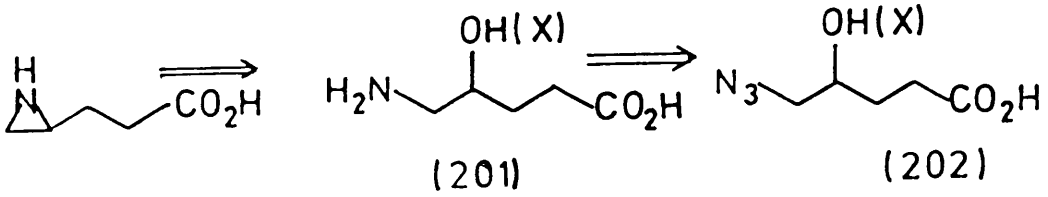


(200)

and (188), prepared from R- and S-glutamic acids, are the chiral starting materials for the construction of the aziridinyl ring in (199) or (200).

Retrosynthetic analysis of either (199) or (200) led to the 2-azidomethyl-alcohol (202), which could be converted into the aziridine ring by a 3-exo-tet process. The 2-azidomethyl-alcohol is available from the hydrolysis of (184) or (188). The aziridine ring would have to be prepared from (202), since the 2-aminomethyl alcohol (201) would cyclise to the δ-lactam (185).[5]



(201) (202)

X - a good leaving group

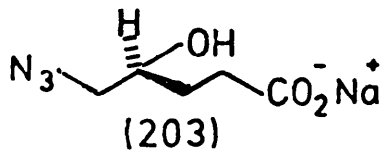
Scheme 29

Tertiary phosphines[21-25] are known to convert 2-azido alcohols to aziridines. Lithium aluminium hydride[26] converts 2-iodo azides

and similar compounds to aziridines. Application of these reagents to our substrates should afford the target molecules (199) and (200).

3.1.2 Results and Discussion

The azidomethyl lactone (184) was treated with one equivalent of sodium hydroxide in boiling methanol for 2 hours to give the (S)-(+)-sodium-5-azidomethyl-4-hydroxypentanoate (203). γ-Butyrolactone has been shown to undergo both acid and basic hydrolysis with acyl-



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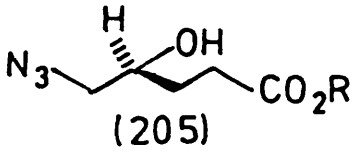

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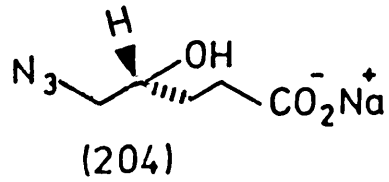

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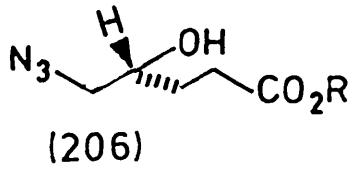


(206)

R = CH_2CH_3

oxygen fission,[20] so cleavage of the acyl-oxygen bond of the lactone (184) should result in retention of configuration in the γ-hydroxy acid formed.

Sodium salts of carboxylic acids react with alkyl halides at room temperature in dipolar aprotic solvents to give high yields of carboxylic esters[28] by the SN 2 mechanism. This procedure was applied to the carboxylates (203) and (204). The reaction was first conducted in dioxane, an inert solvent, at room temperature with ethyl iodide. After twenty hours the reaction mixture was

found to contain two main components by TLC analysis. After work-up and column chromatography, two fractions were isolated. The main fraction was found to be the γ-lactone (184), IR (1770 cm^{-1}), while the minor fraction was found to be the desired product (205). IR (1720 cm^{-1}, 3400 cm^{-1}). This indicated that the esterification which occurred was complicated by subsequent cyclisation to the γ-lactone (184). Apart from the two main components, there were several other spots according to TLC.

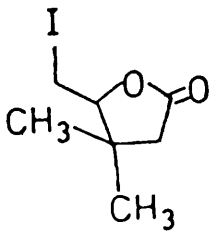
Since dipolar aprotic solvents such as DMF, DMSO and HMPTA are usually employed in this process, DMF was used in the next reaction. The carboxylate (203) was treated with ethyl iodide at room temperature. After twenty hours, aqueous work-up ($H_2O/CHCl_3$) gave an oil which was found to contain two main components according to TLC. The IR spectrum of this mixture showed three absorption bands (1770, 1730 and 3400 cm^{-1}). Column chromatographic separation of this mixture afforded the lactone (184) as the main component with only a trace amount of the desired ester (205). The lactone from the reaction was found to have the S configuration [same optical rotation as that of (184)]. It therefore appeared that the cyclisation proceeded by the SN 2[29] mechanism, the hydroxyl group attacking the ester with elimination of ethanol. This process led to retention of the configuration. The lactone was isolated in 40-50% yield. The desired product (205) was found to be unstable on standing at room temperature for a long period of time. The IR spectrum of this product showed only one carbonyl absorption band at 1730 cm^{-1}. The ^{1}H and ^{13}C NMR spectra were consistent with the structure. The mass spectrum gave the expected ion 188 (M+1).

The same procedure was applied to the R-isomer (204). The carboxylate (204) was treated with ethyl iodide in DMF at room temperature

and stirred for forty hours. Analysis of the reaction mixture still indicated the presence of two components, the lactone and the ester, according to TLC and IR spectroscopy. These were separated by column chromatography after aqueous work-up ($H_2O/CHCl_3$). The main fraction contained the desired product in 60% yield. The IR and NMR spectra were consistent with structure (206). The mass spectrum also gave 188 (M+1). The γ-lactone was also recovered and corresponded to (188). This reaction was carried out on a 0.6 mmol scale. If the reaction was carried out on a much larger scale, the yield decreased and the yield of the γ-lactone increased significantly. Employing non aqueous work-up did not help, since the lactone formation was taking place during the course of the reaction. However, both (S)- and (R)-isomers of the esters (205) and (206) are available from the corresponding carboxylates (203) and (204) in limited amounts.

In order to improve the yield of the esters (205) and (206), other methods leading to their formation from the lactones (184) and (188) were investigated. Alcoholysis of (184) with benzyl alcohol in THF in the presence of sodium hydride was attempted. However, no reaction took place either at room or refluxing temperatures, or over a protracted period of time.

Takano *et al.*[30] have shown that γ-iodomethyl-γ-butyrolactone (207) afford γ,δ-epoxy ester (208) when subjected to methanolysis



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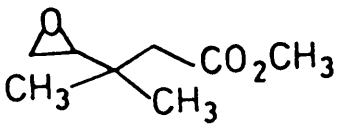
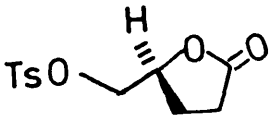


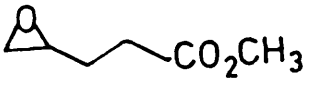
(208)

in the presence of potassium carbonate. It was thus anticipated that the azidomethyl-γ-lactone (184) would give the azidomethyl-γ-hydroxy methyl ester of (203) when treated with methanol under the same conditions. Thus (184) was treated with three equivalents of potassium carbonate in dry methanol at room temperature. No reaction took place, even after five hours. The mixture was then heated under reflux. After one hour, TLC analysis indicated complete reaction of starting material. However, no ester seemed to have been formed. Only the carboxylate salt was isolated, which was an indication that hydrolysis occurred, but esterification had not proceeded.

The tosylate lactone (183), a synthetic equivalent of the iodomethyl lactone (207) used by Takano *et al.*,[30] was subjected to methano-



(183)

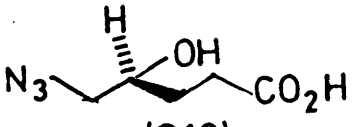


(209)

lysis under the same conditions. After seven hours' stirring at room temperature, TLC analysis showed that the starting tosylate lactone was all consumed. The solvent was evaporated off *in vacuo* at room temperature. Extraction of the residue with organic solvents gave no desirable product at all. It was anticipated that (183) would give the epoxide (209).

In view of these unsuccessful attempts, another approach to solve the problem was sought. Although a free acid liberated from the carboxylate salt (203) might spontaneously cyclise on acidification to give the lactone, an attempt was made to investigate the stability

of this acid across the pH range. So, the carboxylate (203), obtained after hydrolysis of the azidomethyl lactone (184) with sodium hydroxide, was acidified with 2 N hydochloric acid at room temperature to pH 7.0. Extraction of the solution with chloroform or ethyl acetate gave no free acid at all. The pH was then further adjusted to 4.5. At this stage the lactone formation was already taking place. Extraction of the solution with ethyl acetate recovered most of the acid from the aqueous layer. The acid (210) was found to cyclise to the lactone even on standing. In solution, cyclisation occurred rapidly. Treatment of (210) with diazomethane



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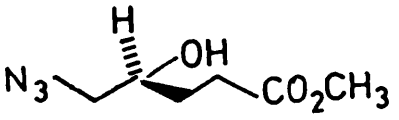
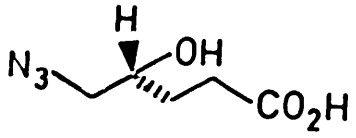


(211)

at 0 °C in ether or ethyl acetate gave, after chromatographic purification on silica gel, a pale yellow oil in 55% yield. This oil corresponded to (S)-(-)-methyl-5-azidomethyl-4-hydroxypentanoate (211). It was found to be optically-active in chloroform, $[\alpha]_D^{25}$ -10.43 (C 1.83, $CHCl_3$). The IR spectrum showed a carbonyl absorption band at 1730 cm^{-I} (C=O), the azido absorption at 2110 cm^{-1} and one at 3480 cm^{-1} (OH). 270 MHz ^1H and ^{13}C NMR gave satisfactory spectra which corrresponded to structure (211). The mass spectrum gave the expected ions at m/z 174 (M+1), 155 ($M-H_2O$), 142 ($M-CH_3O$) and 85 (100%), as a base peak. The R-isomer (212) was similarly prepared from the carboxylate (204) *via* the acid (213). The optical rotation $[\alpha]_D^{25}$ 10.16 (C 1.87, $CHCl_3$), in chloroform was equal and opposite to that of the S-isomer. It also gave spectral data identical to that of the S-isomer. The yield of these esters was modest (50-55%). This



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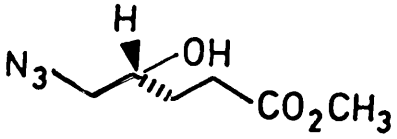
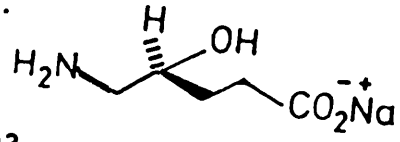


(212)

was attributed to the problem of extracting the free acid from the aqueous layer and to the lactonisation process to yield the lactones (184) and (188). Both lactones could be isolated and separated from the esters by column chromatography on silica gel. On standing over a long period of time, these esters cyclised to lactones.

Catalytic hydrogenation of the carboxylate salts (203) and (204) in methanol at room temperature over 10% palladium on carbon gave the amino acids[27] (214) and (215) respectively. These gave satisfactory spectral data.



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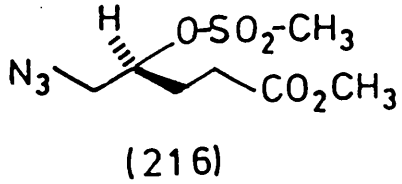

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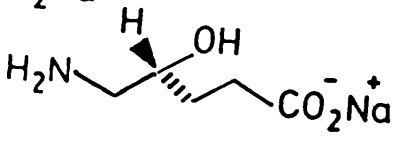
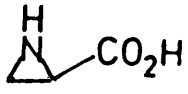


(215)

The hydroxyl group in (211) was activated by converting it to the mesylate ester (216) by reaction with methanesulphonyl chloride in pyridine at 0 °C, and was isolated in 89% yield. The IR spectrum (2100 and 1730 cm^{-1}) contained no absorption band at 3480 cm^{-1} (OH). ^{1}H and ^{13}C NMR spectra were consistent with the structure. The mass spectrum gave the expected ions, m/z 252 (M+1) and 155 ($M-CH_3SO_3H$).

With compounds (211), (212) and (216) in hand, what remained was to convert them to the desired aziridinyl targets. Japanese chemists[31] have prepared *L*-aziridine-2-carboxylic acid (217) as its lithium salt from the corresponding *N*-trityl-*o*-tosyl-*L*-serine benzyl ester. They activated the alcohol in (218) as the 4-methylbenzene-sulphonate ester, followed by heating in base, affording the *N*-trityl-*L*-aziridine-carboxylic acid benzyl ester (219).



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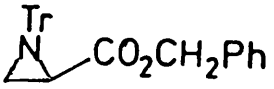

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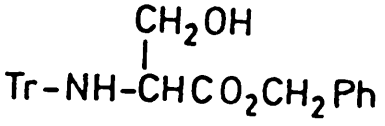

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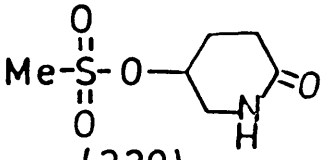


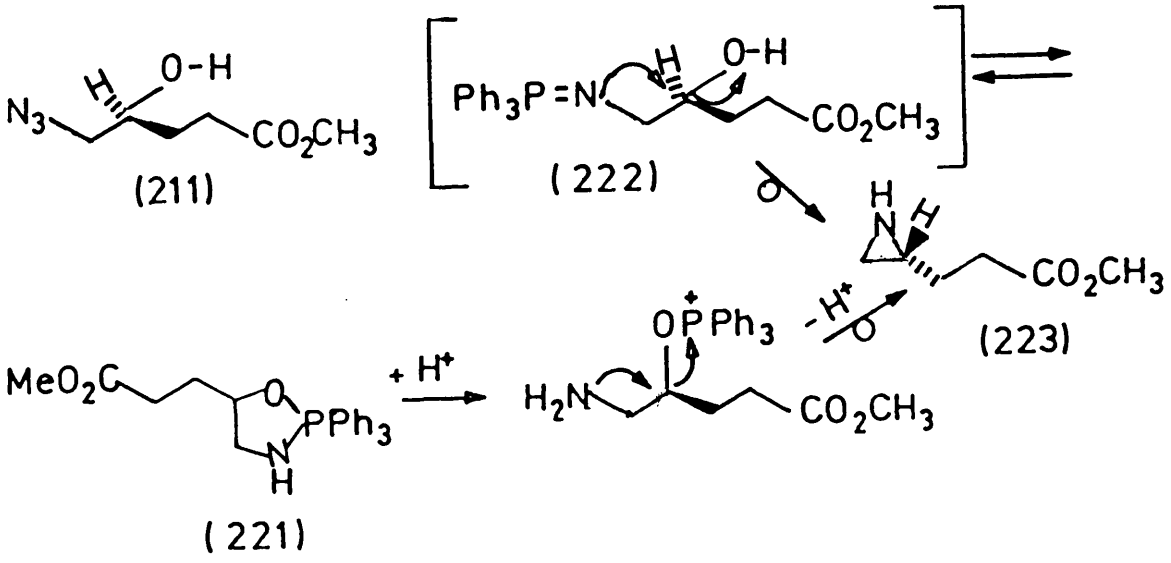
(220)

Unfortunately, catalytic hydrogenation of the azido-mesylate (216) in methanol over 10% palladium on carbon gave the lactam (220),[5] a crystalline product which gave satisfactory spectral data and elemental analysis. It appeared, therefore, that the target molecule could not be prepared by this method. It had to be prepared directly from the azido compounds (211), (212) and (216). Attempts to reduce the azido group in (216) by catalytic hydrogenation in the presence of gaseous hydrogen chloride to yield the amine hydrochloride salt failed. No reaction took place.

The reaction of tertiary phosphines with 2-azido alcohols to form aziridines is well documented.[21-25] These reagents were investigated with substrates (211), (212) and (216). Thus (211) was treated with triphenylphosphine[21,22] in dry acetonitrile at room

temperature or ether at refluxing temperature. Within one hour crystalline triphenylphsophine oxide could be detected. Although most of the oxide could be removed by filtration of an ethereal solution at 0 °C, not all of it could be removed and it seriously contaminated the product. Column chromatographic separation was ruled out, since both the product and the oxide had the same R_f value. Only careful distillation of the mixture afforded the product in an almost pure state. The yield was only 51%.

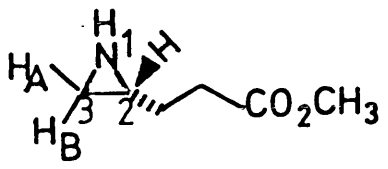
Earlier researchers[21,22] have argued that the mechanism for such an aziridine formation involves reaction at the azide group to give an intermediate (221)[22] or (222),[21] which displaces the hydroxyl or the $OPPh_3$ with inversion (Scheme 30). We therefore assign R-stereochemistry

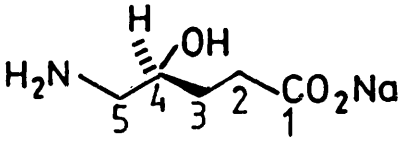


Scheme 30

to the product (223) of this reaction. The structure of compound (223) was determined from the spectral data in comparison with that of the open-chain amino acid (214). Compound (223) had $[\alpha]_D^{25}$ 16.75 (C 4.18, $CHCl_3$), in comparison with the parent compound (211), $[\alpha]_D^{25}$ -10.43 (C 1.83, $CHCl_3$). The 270- MHz ^1H and ^{13}C NMR spectra in $CDCl_3$

allowed rigorous assignment of structure (223). The two methylene protons at C_3 of the aziridine ring were markedly different in chemical shift. Proton H_A, for example, appeared at 1.32 ppm, whereas proton H_B appeared at 1.76 ppm. Each proton coupled to the





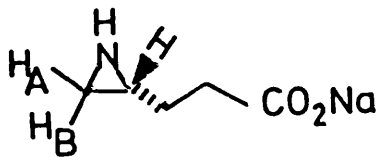
(214)

C_2 proton differently, allowing assignment. As in all aziridines whose NMR spectra have been studied,[32] J_{cis} is always larger than J_{trans}, with J_{gem} being much smaller than the two. Proton H_A at 1.32 ppm had J_{trans} = 2.94 Hz, a typical *trans* coupling. Proton H_B at 1.76 ppm had J_{cis} = 6.41 Hz, a *cis* coupling constant. There was no geminal coupling observed at all for these protons. The H_2 proton appeared at 1.79-2.03 ppm as a multiplet.

In the open-chain amino acid (214) the methylene protons attached to the amino group resonated between 2.53-2.73 ppm as a four-line multiplet, displaying a typical AB system, with J_{AB} = 13.38 Hz and J_{AX} 3.84 Hz. The methine proton at C_4 appeared at 3.53-3.62 ppm as a multiplet. The ^{13}C NMR spectrum of (223) showed that the C_3 ring carbon appeared upfield at 24.93 ppm, with C_2 at 29.29 ppm, whereas in (214) C_5 next to the amino group appeared at 47.34 ppm and C_4 at 71.75 ppm.

The mass spectrum of (223) contained the expected ion at m/z 130 (M+1) with m/z 69 as the base peak. The IR spectrum had an absorption band at 3300 cm^{-1} (NH) with the carbonyl at 1720 cm^{-1}.

Compound (223) was further treated with one molar equivalent of sodium hydroxide in boiling methanol-water (4:1) for 30 minutes to afford the sodium salt of the target molecule (224), sodium-R-(2-aziridinyl)-2-propanoate, in quantitative yield. The 270- MHz

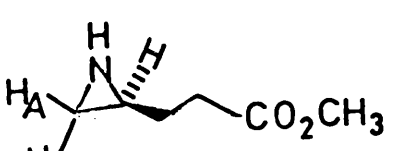


(224)

^1H and ^{13}C NMR spectra in D_2O allowed assignment of structure (224). As in the parent compound (223), the methylene protons at C_3 in the ring appeared at different positions. Proton H_A appeared at 0.625 ppm with J_{trans} = 3.66 Hz, while proton H_B appeared at 1.04 ppm with J_{cis} = 6.04 Hz. Again, no geminal coupling constants could be detected. Correlation of the spectrum with that of the open-chain (214) again illustrated the difference between them. The methylene protons attached to the amino group in (214) displayed an AB system. Spin decoupling experiments further allowed confirmation of structure (224). Selective irradiation of proton H(5a) resontating at 0.54 ppm simplified the spectrum. Proton H(5b) resonating at 0.96 ppm still appeared as a doublet, an indication that it is coupled to the H(4) proton. Proton H(4) itself displayed a quartet, since it is now coupled to proton H(5b) and to the 2H(3) protons. Irradiation of proton H(5b) affected the neighbouring protons and caused proton H(4) to disappear completely. With the irradiation of H(4) and H(5b), proton H(5a) appeared as a singlet. Irradiation of 2H(3) caused the two proton triplets at 1.48 ppm to collapse to a singlet. While proton H(5a) remained

unaffected, the H(4) proton signal became broad. Irradiation of the 2H(2) caused protons 2H(3) to appear as an AB system.

The S-isomers (225) and (226) were similarly prepared from (212). Compound (212) reacted with triphenylphosphine in ether to give (225) in 58% yield. It gave satisfactory spectral data which correlated with those of the R-isomer (223). Proton H_A appeared at 1.38 ppm



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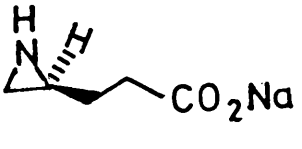


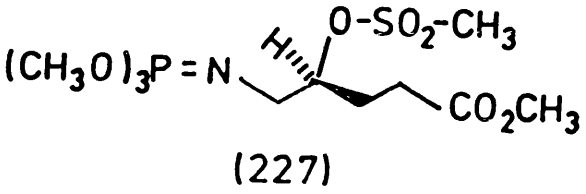
(226)

with J_{trans} = 3.29 Hz, whereas proton H_B was at 1.80 ppm with J_{cis} = 6.41 Hz. Proton H_2 appeared at 2.10 ppm as a multiplet. The mass spectrum gave an ion, m/z 130 (M+1). Hydrolysis of the ester in (225) under the conditions similar to those employed for compound (223) gave the sodium salt of (226) in quantitative yield. The ^1H and ^{13}C NMR spectra were the same as those of the R-isomer (224). Proton H_A appeared at 0.63 ppm with J_{trans} = 3.66 Hz, while proton H_B appeared at 1.04 ppm with J_{cis} = 6.04 Hz. Proton H_2 appeared at 1.27 ppm.

Both isomers (224) and (226) are optically-active. The optical rotation for compound (224) was found to be $[\alpha]_D^{25}$ 5.19 (C 3.85, H_2O), while that of compound (226) was $[\alpha]_D^{25}$ -5.33 (C 3.75, H_2O). The NMR spectrum of compound (226), like the R-isomer (224), was compared with that of the open-chain (215). The methylene protons attached to the amino group in (215) displayed an AB system with J_{AB} = 13.37 Hz.

The reaction of compounds (211) and (216) with trimethyl phosphite[23,24] were investigated. Compound (216) was reacted with

1.5 equivalents of trimethyl phosphite in boiling ether to yield compound (227) after 16 hours in 51% yield as a pale yellow oil. The other possible structure (228) was ruled out, because both



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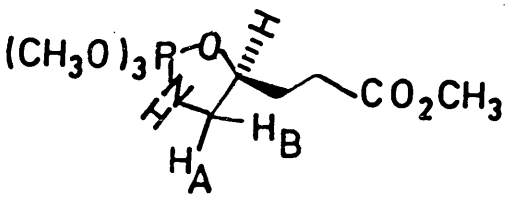
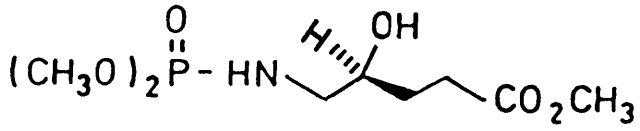


(228)

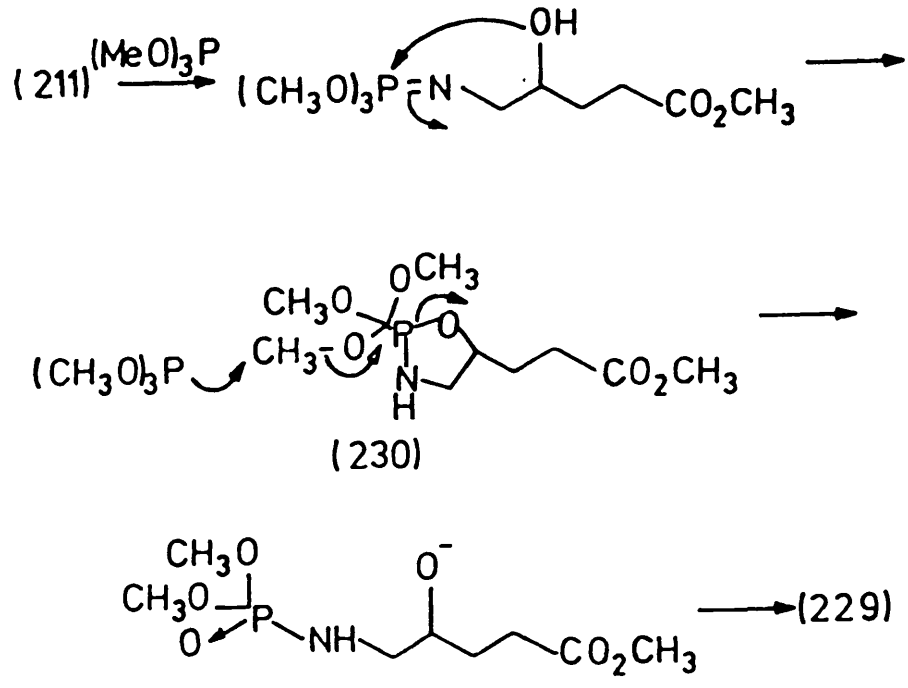
^{1}H and ^{13}C NMR spectra would be different. For example, the methylene protons of cyclic (228) would each have a different chemical shift. The IR spectrum showed a carbonyl absorption at 1730 cm^{-1} and 1430 cm^{-1} (S=O). The 270- MHz ^{1}H and ^{13}C NMR spectra were consistent with structure (227). The mass spectrum gave the expected ions, m/z 348 (M+1), 251 ($M-CH_3SO_3H$), 222 [$M-(CH_3O)_3P$] and 238 a base peak. This compound was found to be stable on standing. An attempt was made to decompose it to the desired aziridine. It was dissolved in toluene and heated under reflux for two hours. TLC and ^{1}H NMR analysis indicated that no change had taken place. Although similar compounds yielded aziridines when treated with alcohols in the presence of sodium hydroxide,[23] this compound was not affected. No further attempts were made to convert it into the desired aziridine compound.

Compound (211) was reacted with trimethyl phosphite[23] in benzene



(229)

under reflux for two hours and yielded (229). The IR spectrum showed two absorption bands at 3390 (OH) and at 3260 cm^{-1} (NH). The carbonyl absorption band appeared at 1740 cm^{-1}. The ^1H NMR spectrum was consistent with structure (229). The mass spectrum indicated a molecular ion at m/z 256 (M+1), 130 [M-$(CH_3O)_2\overset{O}{\overset{\|}{P}}$-$NH_2$] and 238 (M-$H_2O$), $C_8H_{18}O_6PN$, requires M, 255. The mechanism by which this compound is formed is not clear. It probably followed the path depicted below. (211) reacted with trimethyl phosphite to give the cyclic intermediate (230). Since the only nucleophile in the solution is $P(OCH_3)_3$, further attack of $(CH_3O)_3P$ on the carbon of the methoxyl group followed by ring opening of the ring



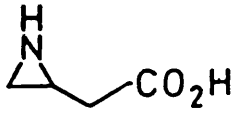
gives (229). Attempts to decompose this compound to the desired aziridine also failed.

Thus, both S- and R-isomers of aziridinyl-2-propanoic acids are available in good yield from R- and S-glutamic acids respectively.

3.2 Approaches to the Synthesis of Aziridinyl Acetic Acid

3.2.1 Introduction

One of the objectives of this project was the synthesis of the unknown aziridinyl acetic acid (176). Two approaches to the synthesis of the aziridine ring in (176) have been explored. The



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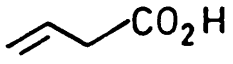


(231)

(232)

first approach involved the use of vinyl acetic acid (231) as the starting point, while the second involved the use of 3-buten-1-ol (232) as the starting point. Many of the routes to aziridines involve intramolecular nucleophilic displacement reactions. The starting materials are often amino alcohols which can be obtained from the ring opening of oxiranes with amines or the reduction of α-amino esters. The OH group is then converted into a good leaving group by reaction with a suitable activating agent and the aziridines are obtained by intramolecular displacement.

Alternative procedures have been developed from olefins. Iodine isocyanate, bromine azide, iodine azide and *N*,*N*-dichlorourethane add to olefins to produce adducts that are precursors to aziridines. In this section, both methods are explored and each approach is discussed separately.

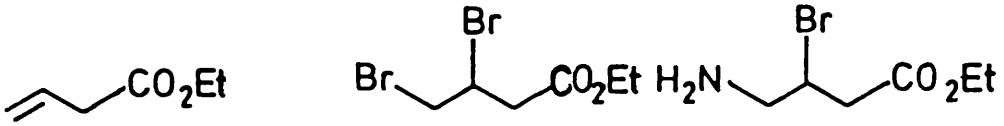
3.2.2 Approaches to Aziridinyl acetic Acid from Vinylacetic Acid

i Vinylacetic acid (231) is a suitable starting point, since it contains the required number of carbon atoms and has the carboxyl group already present in it. The reaction of the double bond in a

protected version of (231) with suitable reagents to yield precursors for an aziridine synthesis should be possible without affecting the carboxyl group. The final removal of the protecting group should afford the target molecule (176).

ii Results and Discussion

The acid (231) was cautiously converted into the ethyl ester (233).[33] This involved the reaction with 1.5 equivalents of thionyl



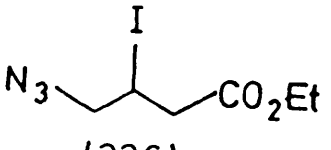
(233) (234) (235)

chloride at 0 °C in ether-DMF (100:1) for three hours to yield the acid chloride which was further treated with sodium ethoxide in ethanol to afford (233) in moderate yield. The reaction of (233) with bromine in dichloromethane at room temperature for two hours yielded the intermediate dibromide (234), which on further reaction with aqueous ammonia in THF at room temperature gave (235), after an exothermic reaction and column chromatography. The IR spectrum contained bands at 3520-3460 cm^{-1} (NH) and at 1735 cm^{-1} (C=O). The 60-MHz ^1H NMR spectrum was consistent with the structure. Although the mass spectrum did not give a molecular ion, it indicated the presence of only one bromine atom with a 1:1 ratio of isotope pattern. Fragment ions at 85 (100%), 165, 167 ($M-CH_3CH_2O$) and 193, 195 ($M-NH_3$) were prominent.

Compounds related to (235) are usually converted into aziridines on treatment with a base by an intramolecular nucleophilic displacement process. Thus (235) was treated with sodium hydroxide[34] in methanol-water (1:2) at room temperature for one hour. Analysis of

the reaction mixture by TLC at this stage indicated complete reaction. The solvent was evaporated at reduced pressure to leave a white solid. The IR spectrum of this solid had a band at 1560 cm^{-1} characteristic of the carboxylate group. There was another band at 1640 cm^{-1}, which is characteristic of the carbon-carbon double bond. The ^1H NMR spectrum in D_2O did not indicate the presence of aziridine ring protons. This solid was dissolved in water and applied to the ion exchange resin, Dowex 50 H^+, column. The column was washed with water to afford a colourless oil, which was subsequently found to be a complex mixture of products.

The reaction of iodine azide with a variety of unsaturated systems to yield precursors for aziridine synthesis is well documented.[35] It adds to olefins in a stereospecific manner in an ionic process. Thus (233) was reacted with iodine azide, generated *in situ* by the reaction of sodium azide and iodine monochloride in acetontrile at 0 °C. After stirring the mixture for 14 hours at room temperature, the IR spectrum of the mixture indicated completion of the reaction, signified by the absence of the absorption band at 1670 cm^{-1} (C=C). After the standard work-up, TLC analysis of the oil obtained revealed the presence of at least six components. The IR spectrum showed the presence of the desired product [2100 cm^{-1} (azido), 1730 cm^{-1}]. Column chromatography of this mixture on alumina afforded only a trace amount of the desired product (236), an oil which quickly darkens on standing,

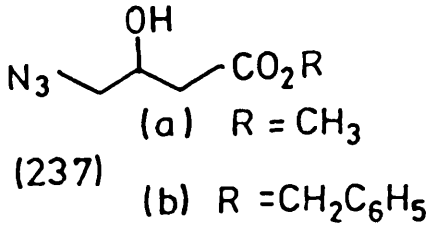


(236)

decomposing into several components. The IR spectrum of this oil gave bands at 2100 cm^{-1} (azido), 1730 cm^{-1} (C=O). The mass spectrum

contained the expected molecular ion m/z 283 (M). Since (236) could not be prepared in a reasonable yield, its reactions were not further investigated. (It had been anticipated that it would react with tertiary phosphines[25] to form the desired aziridine ring.) Compounds of type (236) are unstable since they contain two labile functional groups. Substitution of the iodo functionality at C_3 with the OH group could make it stable since the hydroxyl group is a poor leaving group.

It therefore became apparent that (237) should be prepared from the epoxide (238). The β-iodomethyl-β-lactone (195) was subjected to the epoxidation conditions developed by Takano *et al.*[30] for γ-lactones. This involved the reaction of (195) with methanol in the presence of potassium carbonate to give the epoxide (238). However, methanolysis of (195) under these conditions yielded the α,β-unsaturated ester (239), a ring-opened product of the epoxide which probably resulted from the elimination of the α-methylene proton in



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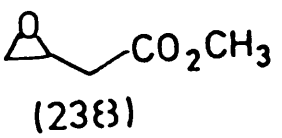

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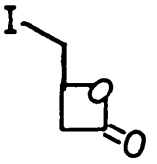
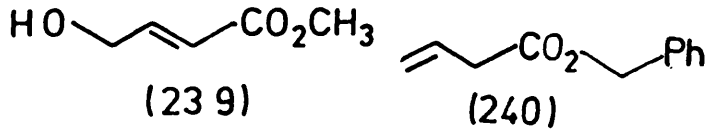


(195)

the epoxide formed, followed by dehydration. The IR spectrum of this compound indicated three distinct absorption bands at 3400 (OH), 1730 (C=O) and at 1650 cm^{-1} (C=C). The 270-MHz ^1H and ^{13}C NMR spectra confirmed the structure. The two olefinic protons displayed an AB system with J_{AB} = 15.75 Hz for the β-proton and J_{AB} = 17.96 Hz for the α-proton which appeared at 6.09 ppm. The β-proton appeared at 7.07 ppm. The OH proton appeared between 3.74-3.75 ppm and disappeared on addition of D_2O. In the ^{13}C NMR spectrum, the α-

and β-carbons appeared at 119.34 ppm and 147.98 ppm respectively, a typical region in which olefinic carbons resonate. The mass spectrum gave m/z 117 (M+!), 98 ($M-H_2O$) and 85 ($M-CH_3O$) corresponding to methyl 4-hydroxybut-2-enoate (239).

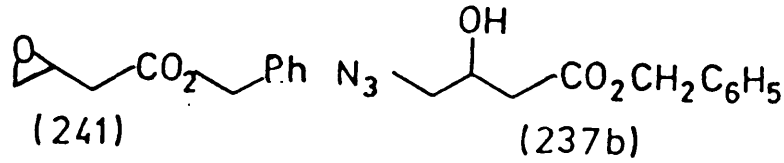
Vinylacetic acid (231) was esterified by reaction with benzyl alcohol using an azeotropic process catalysed by *p*-toluenesulphonic acid in benzene for five days to afford the ester (240) in quantitative yield. Epoxidation of (240) with *m*-chloroperbenzoic acid in



(239) (240)

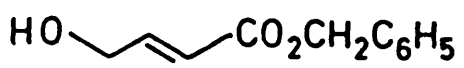
dichloromethane at room temperature for three days yielded the epoxide (241) in 80% yield, after column chromatography on silica gel. Cleavage of this epoxide with the azide ion (NaN_3-DMF) for three hours, gave a yellow oil which was found to consist of a large number of products. Column chromatography of this mixture on silica gel afforded only a trace amount of the desired product (237b). It gave satisfactory spectral data, IR [3400 (OH), 2100 cm^{-1} (azido), and 1730 cm^{-1} (C=O)], mass spectrum [m/z 236 (M+1) and 91 (100%)].

The main fraction from the column was found to be the α,β-unsaturated benzyl ester (242), a hydrolysis product of the epoxide



(241) (237b)

(241). The IR spectrum was identical to that of (239), [3400 (OH),

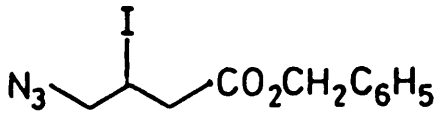


(242)

1700 (C=O) and 1650 cm^{-1} (C=C)]. The 270-MHz ^{1}H and ^{13}C NMR spectra were also identical to those of (239). The α- and β-protons displayed on AB system. The α-proton appeared at 6.12 ppm with J_{AB} = 15.76 Hz and J = 2.2 Hz. The β-proton appeared at 7.03 ppm with J_{BA} = 15.57 Hz and J = 3.89 Hz. The OH proton appeared at 3.21 ppm and disappeared on deuteration. In the ^{13}C NMR spectrum the α- and β-carbons appeared at 119.49 ppm and 148.15 ppm respectively and correlated well with those of (239). The mass spectrum gave the molecular ion at m/z 192 (M+1) and 91 (100%).

Compound (237b) was found to decompose rapidly during column chromatography, which made it impossible to purify it by this technique. Since many azides are known to explode during distillation, no attempt was made to purify this mixture by this method. It became obvious that the impure compound was to be used directly in the next reaction. Thus (237b) was reacted with triphenylphosphine in ether for thirty minutes. TLC analysis of the mixture on alumina or silica gel only indicated a complex mixture of products. The ^{1}H NMR spectrum of the mixture gave no evidence of the presence of the aziridine ring protons.

The reaction of iodine azide[35] with (240) gave the 2-azido iodide (243) in 80% yield. Like the other iodide (236), this compound was found to be unstable on standing. It was purified by flash chromatography on alumina. The structure was determined by virtue of IR and

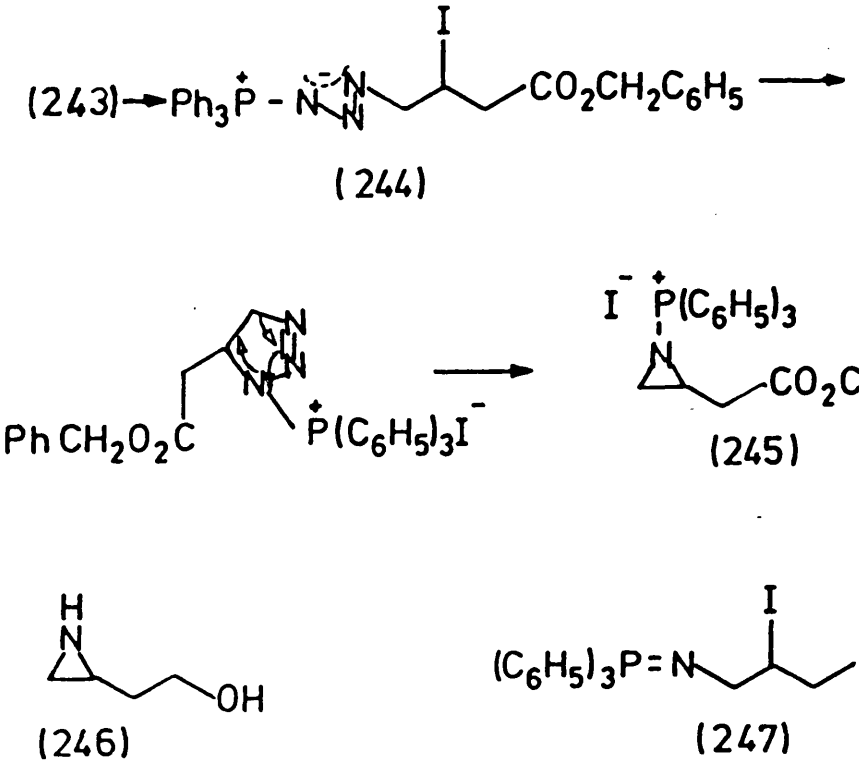


(243)

NMR spectral data only. Due to its instability, no molecular mass measurement was possible. The reaction of this compound with triphenylphosphine and lithium aluminium hydride was investigated.

Compound (243) was reacted with lithium aluminium hydride in ether for 15 hours at room temperature. The mixture was subjected to the standard work-up procedure to afford an oil which was found to consist of several components by TLC analysis on alumina. The ^1H NMR spectrum did not indicate the presence of the aziridine ring protons. Column chromatography yielded benzyl alcohol, a trace amount of starting material and 4-aminobutanol, which resulted from the reduction of the iodo function.[37] The reductive elimination of both the azido and the iodo functions in the form of IN_3,[36] is another side reaction which usually accompanies the aziridine ring formation in this process. Further reactions of the strained aziridine ring, such as polymerisation, probably accounted for the absence of any desired product from the mixture.

Triphenylphosphine usually reacts with 2-iodo alkyl azides by attack on the azide to form *N*-phosphorylated aziridines. It was therefore anticipated that (243) would react with triphenylphosphine to give (244), an intermediate which should give the aziridinyltriphenylphosphonium iodide salt (245). The reduction of this intermediate with lithium aluminium hydride should proceed with P-N

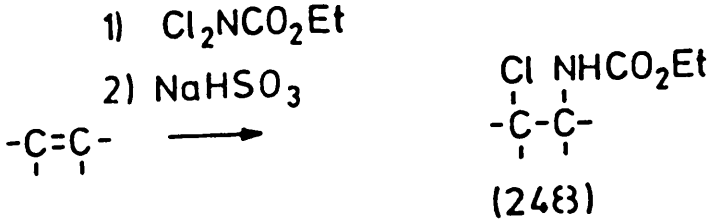


bond cleavage, as well as the reduction of the ester to yield (2-aziridinyl)ethanol (246).

Thus (243) was treated with triphenylphosphine[25] in ether at room temperature. The progress of the reaction was monitored by the IR spectroscopy. After eight hours of stirring the mixture in an atmosphere of nitrogen, all the starting compound was consumed, as evidenced by the absence of the azido absorption band at 2100 cm^{-1}. Analysis of the mixture by TLC both on silica gel and neutral alumina revealed that it was a mixture of several components. The ^1H NMR spectrum of this mixture revealed no presence of the aziridine ring protons of the desired product. However, it was possible that the open-chain isomer (247) could be present. The mixture was therefore treated further with lithium aluminium hydride in ether. After the

usual work-up, a complex mixture was obtained and the ^1H NMR spectrum gave no evidence of the presence of the desired product. The reaction was finally abandoned. Iodine azide is known to be the most versatile of the pseudohalogens,[35] *i.e.*, high selectivity combined with high reactivity, so it was the reagent of choice for a practical aziridine synthesis.

As an alternative to the iodine azide addition methodology, *N*,*N*-dichlorourethane addition reaction was next investigated. The reaction of *N*,*N*-dichlorourethane (DCU) is well studied.[38] Regioselective addition usually occurs with unsymmetrical olefins.[39] Although the adduct (248) is often obtained as a mixture of stereo-

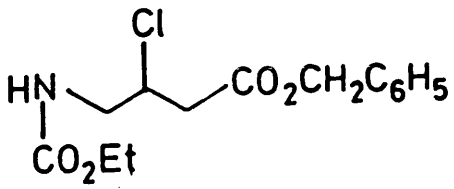


(248)

isomers, there are examples of stereospecific addition to cyclic olefins.[40] DCU adds to 1-olefins to place the nitrogen on the terminal carbon atom. The addition is a free-radical reaction and yields a mixture of stereoisomers.[38b] With terminal olefins where this problem does not exist, the addition is rapid and efficient.

Thus (240) reacted with DCU in refluxing benzene for twenty hours. Subsequent treatment of the cooled solution of this mixture with aqueous sodium thiosulphate gave the chlorocarbamate (249), isolated after column chromatography as a pale yellow oil in 62% yield. The IR spectrum [1720 (C=O), 3440 cm^{-1} (NH)] gave the

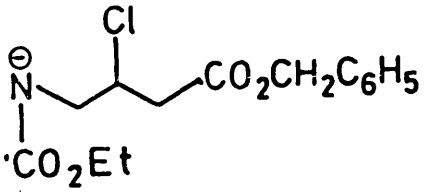
anticipated absorption bands. The 270-MHz ^{1}H and ^{13}C NMR spectra in $CDCl_3$ allowed determination of the structure. The α-protons next to the asymmetric centre displayed an ABX pattern with the β-proton. They appeared at 2.75 ppm and 2.87 ppm with J_{AB} = 16.31 Hz.



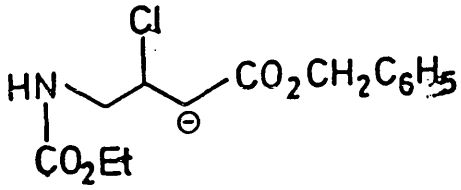
(249)

The γ-protons appeared as a multiplet at 3.49 ppm, since they are also coupled to the NH proton. The β-proton appeared at 4.40 ppm as a multiplet. The NH proton appeared at 5.50 ppm and did not exchange with D_2O. There were two carbonyl carbon atoms in the ^{13}C NMR spectrum; one at 169.64 ppm for the $\underline{C}O_2CH_2C_6H_5$ and the other at 156.73 ppm for the $\underline{C}O_2C_2H_5$. There were also two different carbon atoms bonded to oxygen in the alkoxyl groups. The carbon atom in the benzyloxyl group resonated at 66.83 ppm, while the one in the ethoxyl group appeared at 61.14 ppm. The rest of the carbon atoms appeared in the expected regions. Finally, the high resolution mass spectrometry gave the expected mass value (m/z 299.0852).

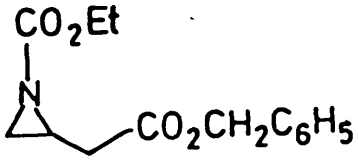
Treatment of (249) with one equivalent of base will generate either the anion (250) or (251). Anion (250) can cyclise to the aziridine (252) by a 3-exo-tet process or to the γ-lactam (253) by a 5-exo-tet process, both of which are favoured. The anion (251) can cyclise to the γ-lactam (254) in a 5-exo-tet favoured process. The aziridine (252) can further undergo a transformation in which



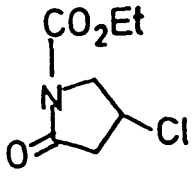
(250)



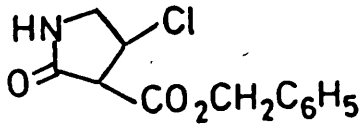
(251)



(252)

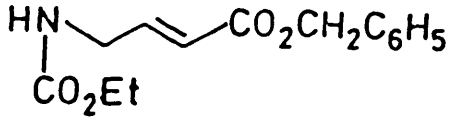


(253)



(254)

the α-methylene proton is eliminated to give (255). It was clear



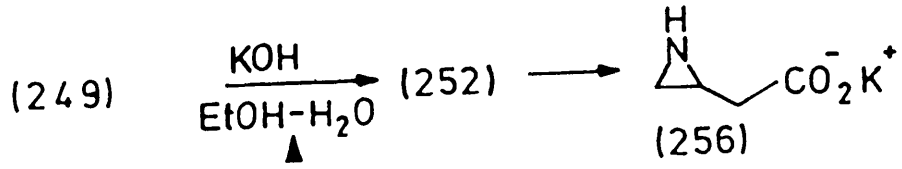
(255)

therefore, that the aziridine formation process would have to compete with the other favoured processes leading to the formation of these products. Also, carbamates of type (249) are known to yield oxazolidones on pyrolysis[38] and so the reaction temperature of the aziridine formation from (249) would have to be closely monitored to prevent this undesirable reaction from taking place. The 2-chloroalkylcarbamates are known to yield aziridines when treated with alcoholic potassium hydroxide.[41] The reaction of (249) with bases was investigated.

The chlorocarbamate (249) was first reacted with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane at 0 °C for 3½ hours. The IR spectrum indicated an absorption band at 1660 cm^{-1} (C=C) and the NH band at 3460 cm^{-1}. Column chromatographic separation of the mixture yielded (255) as a sole product. It was characterised by its spectral data. The IR spectrum contained bands at 3460 (NH), 1770 (br, C=O) and 1660 cm^{-1} (C=C). In the ^{1}H NMR spectrum the α- and β-protons formed an AB system. The α-proton appeared at 5.98 ppm as a doublet of triplets with J_{AB} = 15.76 Hz. The β-proton appeared at 6.94 ppm with J_{BA} = 15.75 Hz and $J_{triplet}$ = 4.76 Hz. In the ^{13}C NMR spectrum, the α-carbon appeared at 120.98 ppm and the β-carbon at 145.28 ppm as expected. The data correlated with those of (239) and (242). The mass spectrum gave the expected molecular ion, m/z 263 (M). The same result was obtained when excess of the base was used.

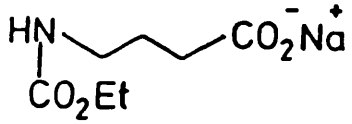
Compound (249) was then reacted with one equivalent of sodium hydride in THF or DMF at 40 °C. After twenty hours the reaction was complete (TLC) and the mixture was found to contain several components. Column chromatography gave (255). Excess of sodium hydride did not change the outcome of the reaction.

Treatment of (249) with alcoholic potassium hydroxide was equally disappointing. It was anticipated that the process would follow the path depicted below to yield the aziridine (256).



One equivalent of potassium hydroxide reacted with (249) in ethanol-water to yield the potassium carboxylate salt in which only the benzyl ester was hydrolysed. When nine equivalents of potassium hydroxide were used, the ethoxyformamido group was still intact too, despite prolonged heating of the reaction mixture over a long period of time. The crystalline salt obtained from the mixture contained no desired product, as indicated by the ^1H NMR spectrum. No further attempts were made to cyclise (249).

Catalytic hydrogenation of (255) over 10% palladium on carbon in THF-water at room temperature in the presence of sodium bicarbonate gave the sodium salt of the ethoxyformamidobutanoic acid (257), whose structure was determined by spectral data.



(257)

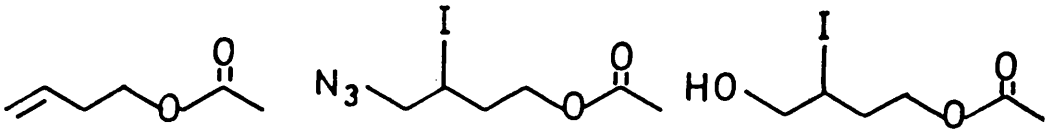
3.2.3 Approaches to Aziridinylacetic Acid from 3-Buten-1-ol

i Introduction

The other possible approach to the aziridinylacetic acid (176) centred on the utilisation of 3-buten-1-ol (232) as a starting material. If the failure to isolate (176) was caused by the elimination of the α-methylene proton to yield the α,β-unsaturated acid derivative (255), the synthesis and isolation of the (2-aziridinyl)-ethanol (246) should be possible at least before the oxidation step to the corresponding acid (176). The double bond in (232) should undergo similar reactions to vinylacetic acid (231) to produce precursors for an aziridine synthesis.

ii Results and Discussion

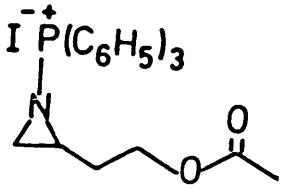
3-Buten-1-ol (232) was reacted with acetic anhydride in dichloromethane in the presence of pyridine at room temperature to yield (258) in quantitative yield. The IR spectrum contained bands at 1740 cm^{-1} (C=O, ester) and 1640 cm^{-1} (C=C). The 270-MHz ^{1}H and ^{13}C NMR spectra were consistent with the structure. The mass spectrum gave the molecular ion, m/z 115 (M+1).



(258) (259) (260)

Compound (258) was then reacted with iodine azide under the same conditions as those employed in the reaction with (240), to give (4-azido-3-iodo)butyl acetate (259) as a colourless oil which darkens on standing in the light. It was isolated in 51% yield after column chromatography on silica gel. The IR spectrum contained two main absorption bands at 2100 (azido) and at 1730 cm^{-1} (C=O). The 270-MHz ^{1}H and ^{13}C NMR spectra were consistent with structure (259). The mass spectrum gave a molecular ion, m/z 284 (M+1) and 241 (M-CH_3O). Another fraction from the column was found, surprisingly, to correspond to (4-hydroxy-3-iodo)butyl acetate (260). The IR spectrum did not contain an absorption band at 2100 cm^{-1}, but now had a band at 3420 cm^{-1} (OH) and at 1720 (C=O, ester). The ^{1}H NMR spectrum allowed determination of the structure. The OH proton appeared at 3.09 ppm and disappeared on deuteration. The rest of the protons appeared in the expected regions. The mass spectrum gave m/z 259 (M+1), 241 (M-H_2O) and 131 (M-HI).

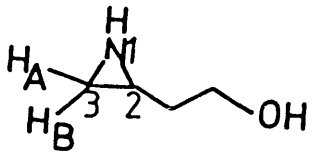
The reaction of (259) with triphenylphosphine[25] should yield the aziridinium salt (261), which, on further reduction with lithium



(261)

aluminium hydride, should yield the aziridine (246). Thus triphenylphosphine was added to the solution of (259) in dichloromethane at 0 °C. The reaction was monitored by IR spectroscopy. Analysis of the mixture after four hours revealed the absence of the starting material, but it was a complex mixture of products. After removal of the unreacted reagent, the solid residue recovered from the solvent was found to contain at least six spots by TLC analysis. Examination of this mixture by NMR spectroscopy did not indicate the presence of the aziridine ring protons. This mixture was further shaken with lithium aluminium hydride in THF at room temperature for 3 days. After the usual work-up, the oil obtained contained crystalline triphenylphosphine and several other products. The NMR spectrum of this mixture did not show the presence of any desired product.

Lithium aluminium hydride[26] reduction of (259) should give the aziridine (246) directly. Thus (259) reacted with lithium aluminium hydride in THF at room temperature for 2 days. After the usual work-up, a colourless oil obtained, was found to be a mixture of several components by TLC analysis. Column chromatography of this oil gave the desired aziridine (246) in 8% yield. The 270-MHz ^{1}H and ^{13}C NMR spectra in $CDCl_3$ allowed determination of the structure.

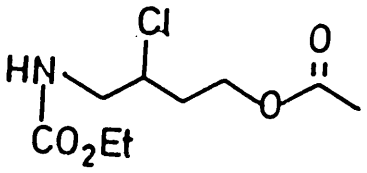


(246)

The ring protons at C_3 displayed the familiar ABX system with proton H(2). Proton H(A3) resonated at 1.47 ppm with J_{trans} = 3.66 Hz. The methine proton H(2) appeared at 2.16 ppm as a multiplet. Proton H(B3) appeared at 1.84 ppm with J_{cis} = 5.87 Hz. The methylene protons next to the asymmetric centre appeared as a multiplet at 1.70 ppm. The protons on the carbon bonded to oxygen appeared at 3.78 ppm as a multiplet. The ^{13}C NMR spectrum also correlated well with that of (223) or (224). The C_3 ring carbon appeared at 23.71 ppm, whereas in (223) it appeared at 24.93 ppm. The C_2 appeared at 28.02 ppm in (246) and at 29.29 ppm in (223). The mass spectrum gave a molecular ion at m/z 86 (M-1) with a base peak at 56 ($M-CH_2=\overset{+}{O}H$).

Another fraction from the column gave 4-aminobutanol as the main product. With the (2-aziridinyl)ethanol in hand, the target molecule (176) should be available after oxidation of the alcohol in (246).

The reaction of (258) with DCU[38] was also investigated. Thus (258) reacted with DCU in refluxing benzene for twenty hours to yield the chlorocarbamate (262) in 89% yield. It gave satisfactory spectral data: IR [3340 (NH), 1720-40 (br, C=O), and 760 cm^{-1} (C-Cl)]; mass spectrum [m/z 238 (M+1), 202 (M-HCl), 192 ($M-C_2H_5O$), and 178 (M-



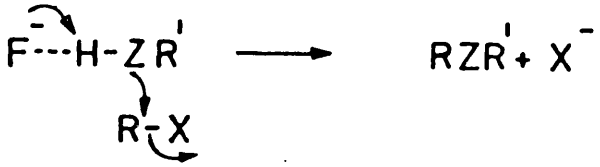
(262)

CH_3CO_2H)] and ^1H and ^{13}C NMR spectra were consistent with the structure.

The reaction of (262) with bases was investigated. Like compound (249), (262) was treated with two equivalents of DBU in chloroform at room temperature. After 75 hours, TLC analysis showed that the starting material was still unchanged. The mixture was then heated under reflux for 17 hours, but this did not affect the reaction. The starting material was recovered. The unreactive nature of (262) towards DBU is not clear, since this reagent is known to promote dehydrohalogenation of alkyl halides.[42]

The reaction of (262) with sodium hydride was also investigated. Thus (262) was treated with sodium hydride in DMF. After stirring the mixture for 40 hours, TLC analysis showed that all the starting material was consumed. The usual work-up gave an oil which was found to be a complex mixture by TLC analysis. No desired product was detected from this mixture.

The ability of the fluoride anion to act as a base in organic reactions is extensively studied.[43] It is known to increase the electron density on the heteroatom to which hydrogen is bonded, thereby producing the fluoride-protic-H-bonded complex which plays the role of the reactant nucleophile. It was therefore anticipated that (262) could cyclise to the desired aziridine ring mediated by fluoride ion. Thus the chlorocarbamate (262) was treated with two



equivalents of caesium fluoride in THF in the presence of benzyltriethylammonium chloride. After stirring the mixture for 16 hours, TLC analysis revealed a complex mixture of products. Close examination of the oil obtained, after work-up, by the ^{1}H NMR gave no evidence of any presence of the desired product.

The chlorocarbamate (262) was finally treated with potassium hydroxide in ethanol-water (40:1) at 50 °C for two days. The solvent was evaporated *in vacuo* to dryness. Extraction of the solid residue with chloroform gave an oil which, by TLC analysis on silica gel consisted of at least 10 spots. On neutral alumina there was at least one main spot which could be separated from the complex mixture. The desired product was separated from this mixture by column chromatography on alumina in 23% yield. The 270-MHz ^{1}H and ^{13}C NMR spectra allowed determination of the structure of this oil. The spectra correlated well with that prepared from the azido iodide (259). The two protons at position C_3 in the ring had different chemical shifts. One appeared at 1.46 ppm as a doublet with J_{trans} = 3.66 Hz and the other at 1.83 ppm with J_{cis} = 6.05 Hz. The proton at C_2 in the ring appeared 2.15 ppm as a multiplet. The ^{13}C NMR spectrum was also identical to that prepared from (259). The C_3 carbon of the ring appeared at 23.99 ppm, while C_2 appeared at 28.01 ppm. The mass spectrum gave the expected molecular ion at m/z 86 (M-1) and 56 (M-CH $=\overset{+}{O}H$) corresponding to the (2-aziridinyl)ethanol (246).

The next task was to oxidise (246) to get the target molecule (176) with preservation of the ring. There are no reported literature procedures involving the oxidation of aziridinyl alcohols into the corresponding aziridinylcarboxylic acids. Two attempts were made to oxidise (246). The first involved the use of neutral or basic potassium permanganate and the second involved the reaction with silver carbonate on celite.

A. The Fetizon oxidation

It has been found that silver carbonate on celite in boiling benzene is a neutral oxidising agent which transforms primary or secondary alcohols into aldehydes or ketones in high yield.[44] It was also later found that it converts primary 1,4-, 1,5- and 1,6-diols into the corresponding lactones in high yield.[45] We therefore assumed that (246) would give the acid (176) when subjected to the Fetizon oxidation process. Thus (246) was reacted with silver carbonate on celite in boiling benzene for three days. After filtration of the inorganic salts and evaporation of the solvent *in vacuo*, the dark yellow oil obtained was analysed. TLC analysis on alumina was impossible because of streaking. On silica gel, at least 8 spots were visible. The IR spectrum of this mixture showed two absorption bands at 1710 (C=O) and another at 1640 cm^{-} (C=C). The NMR spectrum of this oil showed that the ring was also destroyed.

B. Potassium permanganate

The aziridine (246) was treated with potassium permanganate in acetone at room temperature for five days. After removal of the solvent *in vacuo*, the residue was taken up in water and the insoluable material was filtered through celite. Evaporation of the solvent left white crystals. The IR spectrum of these crystals revealed two

absorption bands at 3400 (NH) and at 1560 cm^{-1} ($CO^{-}K^{+}$). The ^{1}H NMR spectrum only showed ring opened products. The ^{13}C NMR spectrum indicated the presence of the carboxylate group at 181.31 ppm. TLC analysis of these crystals on silica gel using methanol-water as solvent indicated a complex mixture of products. No further attempts were made to oxidise (246) due to lack of time.

Thus (2-aziridinyl)ethanol (246), a precursor to (2-aziridinyl)-acetic acid (176) is available from 3-buten-1-ol (232) in reasonable yield either *via* the 2-azido iodide (259) or *via* the 2-chlorocarbamate (262).

CHAPTER 4

ENZYME INHIBITION AND RECEPTOR BINDING STUDIES *IN VITRO*

The enantiomers S- and R-(−)-5-aminomethyl—butyrolactones (186) and (187) respectively and racemic (R,S)-4-aminomethyl—butyrolactone (175) and the enantiomers S- and R-(−)-(2-aziridinyl)-2-propanoic acids (226) and (224) respectively, were tested both for inhibition of GABA-T and binding to GABA receptors *in vitro*. GABA-T was isolated from locust *Supraoesophageal ganglia*. Receptor binding studies were also performed on receptor sites in locust *Supraoesophageal ganglia*. These assays were carried out by G.G. Lunt *et al.*[46]

4.1 GABA Receptor Binding Studies

4.1.1 Introduction

Ligands which compete with GABA for its binding site on the receptor can be characterised by their IC_{50}, which is the concentration of ligand required to depress by 50% the maximum specific binding of [^{3}H]-GABA. Thus a plot of % specific binding against $-\log_{10}$ ligand concentration will generate a curve from which the IC_{50} can be determined.

Under certain defined conditions, the IC_{50} for a ligand approximates to the equilibrium dissociation constant for binding for that ligand, K_i. The experimental conditions for $IC_{50} \approx K_i$ for a particular ligand are related to both the concentration of radiolabelled test ligand and to the concentration of receptor sites. As the concentration of the radiolabelled ligand increases, the difference between the IC_{50} of the unlabelled displacing ligand and its K_i progressively increases at a fixed receptor concentration according to:

$$K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_d}} \qquad (1)$$

where [L] is the concentration of the radiolabelled ligand;

K_d is the equilibrium dissociation constant for the radiolabelled ligand.

The concentration of receptor sites can also influence the relationship between IC_{50} and K_i.[47]

At equilibrium:

$$IC_{50} = n(K_d + L_t + R_t - \frac{3}{2} RL) = K_i + n(L_t + R_t - \frac{3}{2} RL) \qquad (2)$$

where L_t = total concentration of radiolabelled ligand

R_t = total concentration of receptor sites

RL = concentration of receptor-ligand complex formed in the absence of unlabelled ligand

n is a fraction of K_d.

From equation (2):

$$K_i = nK_d \qquad (3)$$

Therefore, if $(L_t + R_t) \ll K_d$ then

$$IC_{50} \approx K_i \qquad (4)$$

Practically speaking, the concentration of receptor sites should not exceed (a) 10% of the K_d for the labelled ligand and (b) the concentration of the radiolabelled ligand should not be more than 25% of the concentration of the unlabelled ligand giving 50% displacement of binding.

In our case, point (a) is easily satisfied since the K_d for [^{3}H]-GABA is 30 nM, while the concentration of receptor sites is 30 pM. Condition (b) is not always attainable.

4.1.2 Results and Discussion

The title compounds (186), (187), (175), (224) and (226) were tested for their ability to displace [^{3}H]-muscimol from the GABA receptor sites on the locust *Supraoesophageal ganglia*. Both the 4-aminomethyl—butyrolactone hydrobromide (175) and the (S)-(+)-5-aminomethyl—butyrolactone hydrobromide (186) had no effect on the binding of [^{3}H]-muscimol to GABA receptor up to the final concentration of 1 mM. The (R)-(-)-5-aminomethyl—butyrolactone hydrobromide (187) was found to be as effective as GABA at displacing [^{3}H]-muscimol from the GABA receptor.

While the (S)-(+)-(2-aziridinyl)-2-propanoic acid (226) had no effect on the binding of [^{3}H]-muscimol on the GABA receptor, the (R)-(-)-(2-aziridinyl)-2-propanoic acid (224) displaced [^{3}H]-muscimol from the receptor at the concentration of 400 μM. It appeared, therefore, that the lactone (187), of restricted conformation linking C_1-C_4 is more active than the aziridinyl propanoic acid (224) also of restricted conformation linking C_4-C_5.

4.2 Inhibition of 4-Aminobutyrate: 2-oxoglutarate aminotransferase, EC 2.6. 19, (GABA-T)

4.2.1 Introduction

The isolation and purification of GABA-T from locust *Schostocerea gregaria* is described by Jeffreys *et al.* (in press). Inhibition studies were undertaken with compounds (186), (187), (175), (224) and (226) *in vitro*.

4.2.2 Results and Discussion

The compound under study was incubated with a known constant amount of the enzyme (GABA-T) in a constant volume of buffer:

50 mM bicine, pH 8.5. However, the results were not very encouraging. The (R)-(-)-5-aminomethyl——butyrolactone (187) had no effect at all on the enzyme. The corresponding S-isomer (186) and racemic 4-aminomethyl——butyrolactone (175) were found to be poor competitive inhibitors with very high K_i values. Neither isomers of (224) and (226) were found to inhibit GABA-T up to a final concentration of 1 mM.

PART B

APPROACHES TO TABTOXININE-β-LACTAM

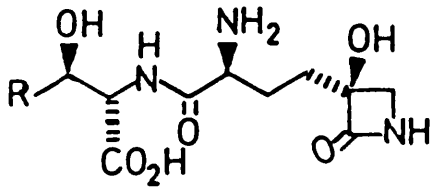
CHAPTER 5

APPROACHES TO TABTOXININE-β-LACTAM

5.1 General Introduction

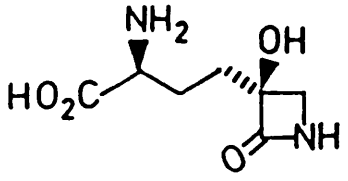
5.1.1 History and Biochemistry

Three closely related phytopathogenic exotoxins have been isolated from wildfire-disease-infected tobacco (*pseudomonas tabacci*), tabtoxin (263), [ser^2]-tabtoxin (264) and tabtoxinine-β-lactam (265).[1] Tabtoxin (263) is a dipeptide exotoxin produced by *pseudomonas tabacci*, the organism responsible for the wildfire



(263) R = CH_3

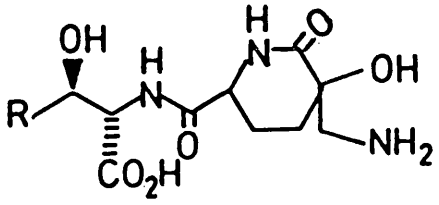
(264) R = H



(265)

disease of tobacco plants.[2] When hydrolysed by peptidases *in vivo*, this exotoxin releases tabtoxinine-β-lactam (265), which inhibits glutamine synthetase in the photorespiratory nitrogen cycle, causing chlorosis and death of the tobacco plant.[3] As the dipeptide (263) itself does not inhibit purified glutamine synthetase *in vitro*,[3c] the amino acid (265) is considered to be the active moiety of (263) and hence the actual toxin of wildfire disease. This inhibition is the result of tight binding of tabtoxinine-β-lactam (265) to the enzyme.[1a] In addition to being a photorespiration inhibitor, the toxin (265) has fungicide, algicide and mammalian toxicity.

Although wildfire-disease was first reported in 1917, the structure of the toxin (263) was only elucidated in 1971, [a] due to its chemical instability. It was found that both toxins (263) and (264) on standing undergo a facile intramolecular transacylation to the stable inactive δ-lactam-isotabtoxin (266).



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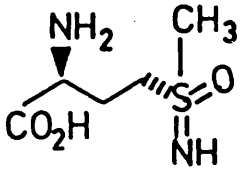
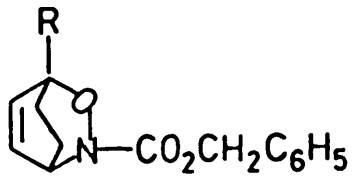


(267)

The inhibition of glutamine synthetase by *L*-methionine-S-sulphoximine (267) and related compounds has previously been documented and extensively studied.[4] However, the detailed mechanism of glutamine synthetase inhibition by tabtoxinine-β-lactam attracts current interest and therefore a synthetic approach to the toxin (265) and its analogues is of increasing importance.

5.1.2 Synthesis of Tabtoxin

Tabtoxin (263) was finally synthesised twelve years after the elucidation of its structure, by Baldwin and co-workers.[5] The key step of their synthesis was an elegant regiospecific cyclo-addition of an acylnitroso compound to a cyclohexadiene. This single step defined the stereochemical relationship between C-3 and C-7. Benzyl nitrosoformate, generated *in situ* from *N*-benzyloxycarbonyl hydroxylamine and tetraethylammonium periodate in dichloromethane, reacted with ethyl cyclohexa-1,3-diene carboxylate to yield the single regio-isomer (268). The regiochemistry was confirmed by hydrogenation



(268) R = CO_2Et

(269) R = CH_2OH

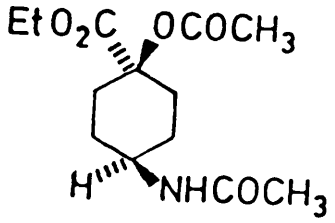
(270) R = CHO

(271) R = $CH_2NHCH(4\text{-}CH_3OC_6H_4)_2$

(272) R = CH_2NH_2

(273) R = $CH_2NHCOCH_2Cl$

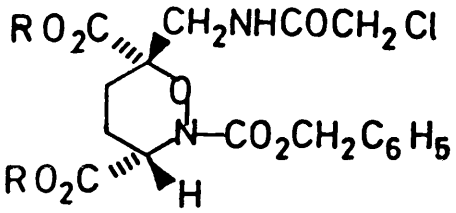
and acetylation to 274, in which ^1H NMR showed a doublet for the amide proton at δ 5.36. The ester (268) was reduced to the alcohol (269) with sodium borohydride and oxidised to the aldehyde (270) using DCC-DMSO in pyridine/trifluoroacetic acid. Direct reductive



(274)

amination resulted in dialkylamine formation. Thus (270) was converted to (271) with 4,4'-dimethoxydiphenylmethylamine and reduced with sodium cyanoborohydride, followed by deprotection with TFA and anisole to the amine (272). Chloroacetylation gave (273). The double bond was then oxidatively cleaved using potassium permanganate in water-benzene with tetrabutylammoniumbisulphate yielding the

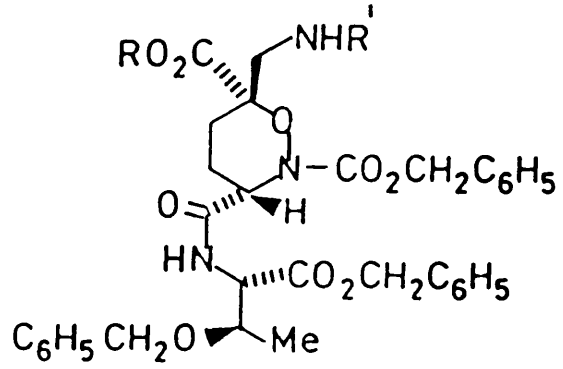
racemic diacid (274). The dipivaloyl mixed anhydride (275) was prepared by addition of two equivalents of pivaloyl chloride and



(274) R = H

(275) R = CO C(CH$_3$)$_3$

triethylamine (2 equivalents). The mixed anhydride (275) was reacted *in situ* with *O*-benzyl-*L*-threonine benzyl ester to give (276) resulting from selective attack at the less hindered of the two carbonyl groups, as a mixture of diastereoisomers. Separation of these isomers was effected by conversion to the crystalline benzyhydryl esters by treatment with diphenyldiazomethane. The ester (277) was crystallised from ethyl acetate and the other from ether. The ester (277) was deprotected with trifluoroacetic acid to the monoacid (276). Dechloroacetylation with thiourea gave the amino



(276) R = H

R' = COCH$_2$Cl

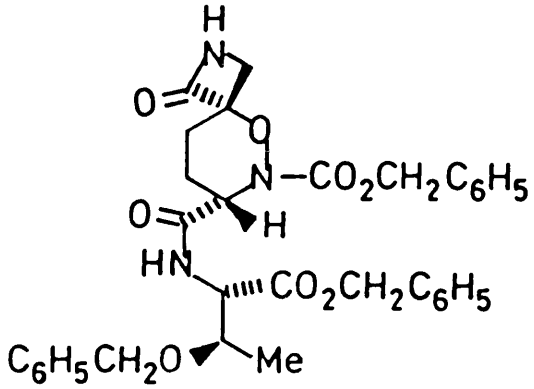
(277) R = CH (C$_6$H$_5$)$_2$

R' = COCH$_2$Cl

(278) R = H

R' = H

acid (278). (278) was cyclised using 2,2'-dithiodipyridine-triphenylphosphine-acetonitrile to give the spiro β-lactam (279). Catalytic hydrogenolysis of (279) afforded tabtoxin (263). The



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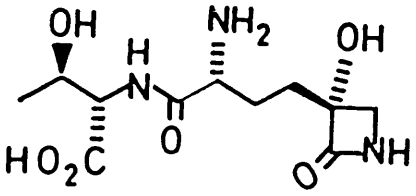
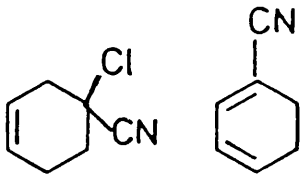


(280)

diastereoisomer (280) prepared from the other diastereoisomer of (276) showed virtually no biological activity.

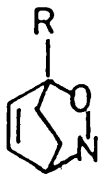
5.1.3 Synthesis of Tabtoxinine-β-lactam

Tabtoxinine-β-lactam (265) was also synthesised by Baldwin and co-workers.[6] Thus, 2-chloroacrylonitrile was treated with butadiene in a sealed tube to give cyclohexene (281). Dehydrochlorination of (281) was effected by heating in pyridine at reflux



(281)

(282)

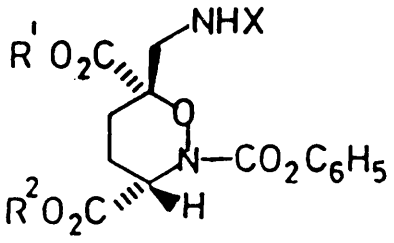


(283) R=CN

(284) R=CH_2NH_2

(285) R = $CH_2NHCO_2Bu^t$

to give the cyano-diene (282). The cyclo-addition of diene (282) to benzyl nitrosoformate (generated *in situ* from benzyl *N*-hydroxy-carbamate and tetraethylammonium periodate in dichloromethane) gave (283) as a single regioisomer. Reduction of the nitrile function in (283) with $NaBH_3(OCOCF_3)$ reagent (generated from sodium boro-hydride and trifluoroacetic acid) gave the primary amine (284). Subsequent protection of this amine with *t*-butyloxycarbonyl group gave (285). Oxidative cleavage of the double bond using potassium permanganate gave the diacid (286). The key differentiation of the two carboxyl groups of (286) was successfully achieved by decarboxyl-



(286) $R'=R^2=H$. $X=CO_2Bu^t$

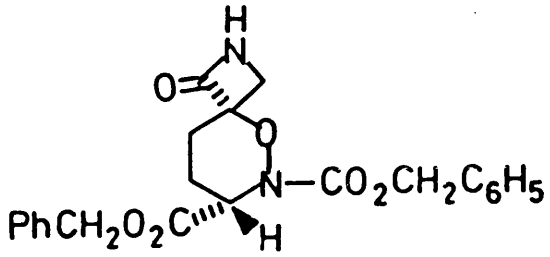
(287) $R'=H$, $R^2=CH_2C_6H_5$, $X=CO_2Bu^t$

(288) $R'=R^2=CH_2C_6H_5$, $X=CO_2Bu^t$

(289) $R'=X=H$, $R^2=CH_2C_6H_5$

ative esterification with benzyl chloroformate and pyridine in di-chloromethane to yield monoester (287) along with a small amount of diester (288). Removal of the primary amino protection of (287) with formic acid gave the amino acid (289). Cyclisation to the β-lactam was achieved by use of Ph_3P-$(PyS)_2$-CH_3CN to yield spiro β-lactam (290). Complete deprotection and reductive cleavage of the N-O bond of (290) was effected in one step by catalytic hydro-genation to afford (±)-tabtoxinine-β-lactam (265). The synthetic sample was identical to a sample isolated from *P. tabacci* and was

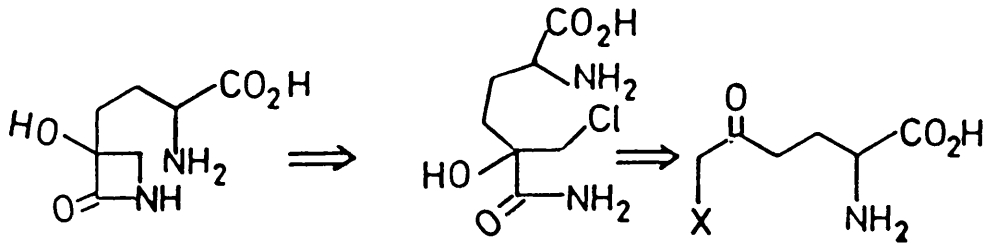
an active glutamine synthetase inhibitor, *in vitro* and *in vivo*.



(290)

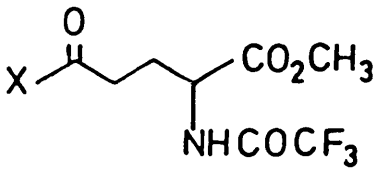
5.1.4 Synthesis of Tabtoxinine-β-lactam Precursors

Precursors of tabtoxinine-β-lactam (265) have been synthesised by C. Smith in our laboratories.[7] Two approaches were utilised in his strategy. The first was the linear approach which involved building the β-lactam moiety at the γ-terminus of glutamic acid. Retrosynthetic analysis led to the α-haloketone which could further be converted into the β-lactam. In this approach the stereochemistry

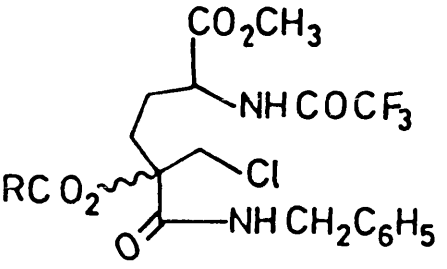


(291) X= Cl

at C_3 was not controlled and the diastereoisomers were separated by using HPLC.

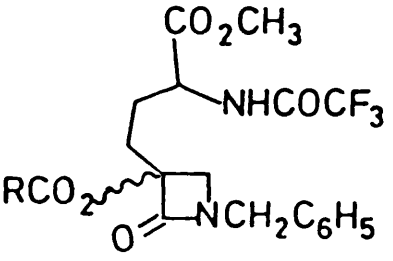


(292) a) X = OH
b) X = Cl



(293)

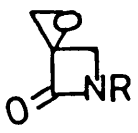
a) R = CH_3
b) R = C_6H_5



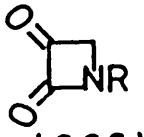
(294)

Thus, the acid (292a), prepared by the method of Ellis *et al.*,[8] was converted to the α-chloroketone (291) *via* the acid chloride (292b). This involved the reaction of (292b) with diazomethane and gaseous hydrogen chloride. The amide (293) was available from the Passerini reaction between (291) and one equivalent each of carboxylic acid and benzyl isocyanide. Cyclisation to the β-lactam (294) was accomplished by treatment with caesium fluoride and a catalytic amount of benzyltriethylammonium bromide in refluxing THF. Racemic mixture of (294) was separated by using HPLC. Deprotection of (294) was not achieved at all.

The second approach involved the construction of the β-lactam ring separately, and the preparation of the side chain. Two β-lactam rings were constructed, the spiroepoxide (295) and the azetidin-2,3-diones (296). It was anticipated that these reagents would react with anions of α,β-unsaturated amino acids (297) and (298) to yield

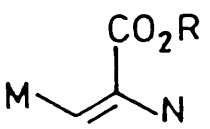


(295)

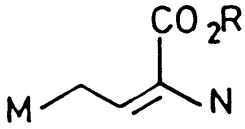


(296)

the tabtoxinine-β-lactam precursors, deprotection of which would furnish the target molecule.



(297)



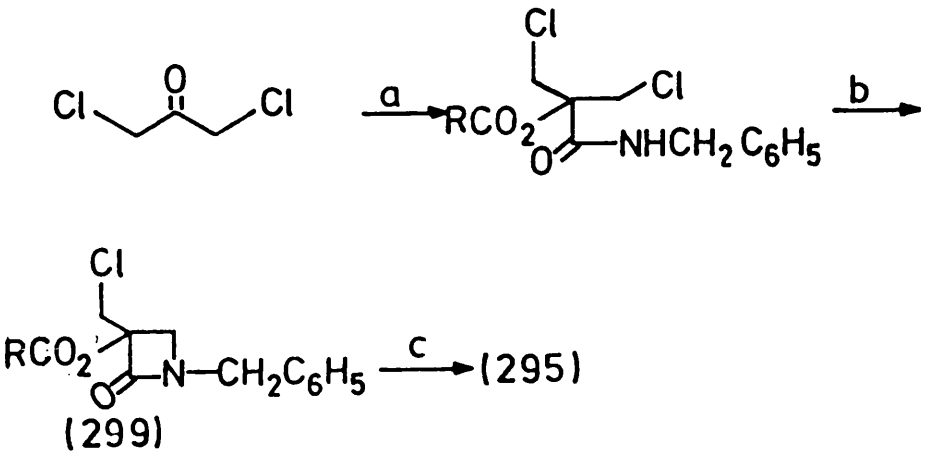
(298)

M = Li, Cu, LiRCu etc

N = N_3, $NR'R''$ etc

The spiroepoxide (295) was prepared according to Scheme 31, a method developed by Sebti and Foucard.[9] Treatment of (299) with lithium hydroxide in THF afforded the epoxy β-lactam (295).

The azetidin-2,3-dione (296) was prepared from *N*-(2,3-dichlorophenyl)-3-methylene azetidin-2-one by oxidation with potassium permanganate and sodium periodate in *t*-butanol/water. The side chain (298) of tabtoxinine-β-lactam was not possible to prepare. In all



Reagents: a, $PhCH_2NC/CH_3CO_2H$ or $PhCO_2H$, b, $CsF/BnEt_3\overset{+}{N}\overset{-}{Br}$, c, LiOH/THF

Scheme 31

systems investigated, the reactive intermediates were either too reactive to yield the desired products, since complex mixtures of products resulted, or too unstable and therefore decomposed before reacting with the electrophiles in solution.

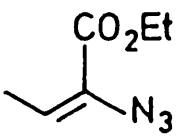
5.1.5 Proposed Synthesis

The proposed synthetic target in this project was tabtoxinine-β-lactam (265). Based upon initial studies in our laboratories,[7] the linear approach which afforded tabtoxinine-β-lactam precursors was further explored. In particular, we were concerned with finding appropriate protecting groups since in our earlier studies,[7] inappropriate protection methods had been applied. Also other approaches to the side chain of the target molecule were investigated.

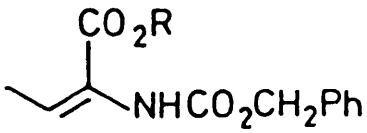
5.2 Approaches to the Side Chain of Tabtoxinine-β-lactam

5.2.1 Introduction

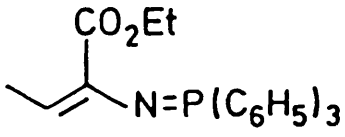
As stated in the introduction, C. Smith[7] synthesised β-lactams (295) and (296). These reagents would react with (297) and (298), thereby building the tabtoxinine-β-lactam skeleton. A series of α,β-unsaturated amino acids (300-304) as either dienolates or enamides were investigated. The anions generated from these reagents were exposed to suitable electrophiles to yield alkylation products, presumably at the γ-position. If successful, the anions would have been reacted with the β-lactams (295) and (296) to yield the target molecule after deprotection.



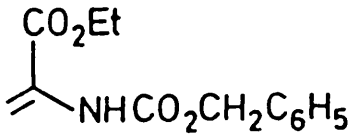
(300)



(301) R = H, CH_3



(302)



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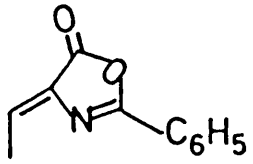

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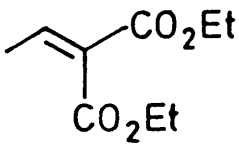


(305)

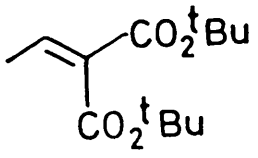
The anion of (300) could not be generated, due to the labile azido group which liberated nitrogen. Attempts to utilise the anions from (302-304) were unsuccessful.

The anion of ethyl 2-carboethoxybut-2-enoate (305) was generated, but gave the 2-alkylation product when trapped with benzyl bromide. Other electrophiles, such as benzaldehyde, and methyl iodide, gave complex mixtures of products.

In continuation of the search for a suitable nucleophilic reagent which would successfully react with the β-lactam (295) or (296) to yield the target molecule, it was decided to investigate the anions of other malonic acid derivatives such as (306) and (307). If successful, they could then be converted to amino acids *via*, for example, a Curtius rearrangement of an acyl azide.

5.2.2 Preparation of *t*-Butyl 2-carbo-*t*-butyloxybut-2-enoate and 2-Ethylidine-2,2-dimethyl-1,3-dioxan-4,6-dione: Generation of their Anions

Compound (307) has been synthesised by P. Margaretha[10] from 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (308). This acid reacts with a variety of simple aliphatic aldehydes and ketones to



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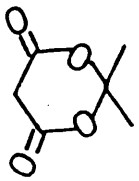
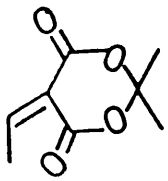


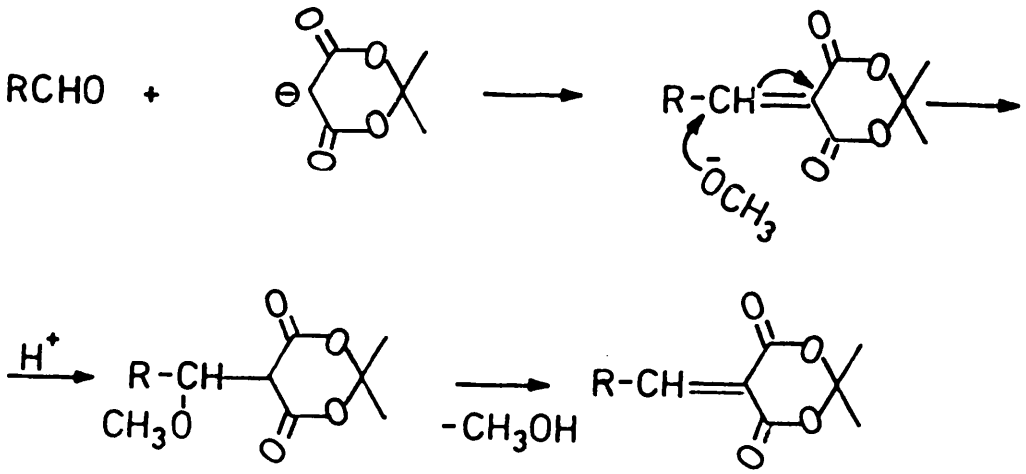
(308)



(307)

yield the 2:1 condensation products resulting from the Michael addition of the acid to the initially formed α,β-unsaturated compound.[11] Margaretha,[10] however, prevented this Michael reaction of the active methylene compound (Meldrum's acid) with the initial

product, by performing the aldol condensation in methanol (see Scheme shown below). The CH_3O^- is a competitive nucleophile in the Michael



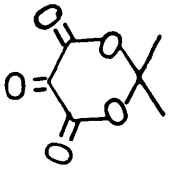
addition. The product so formed is easily decomposed with acid to yield the α,β-unsaturated product.

Applying the same procedure[10] to make compound (307) failed in our hands and we had no other option but to search for another method. A number of bases, such as triethylamine, sodium hydride and di-isopropylamide were used to generate the anion of (308), which was subsequently trapped with acetaldehyde. When triethylamine was used in THF at lower temperature followed by silylation with *t*-butyldimethylsilyl chloride and subsequent addition of acetaldehyde, a complex mixture of products was obtained after work-up, with no desired product detected.

The reaction of (308) with sodium hydride did not promote the condensation between the anion and acetaldehyde, since only the starting compound was recovered.

Lithium di-isopropylamide (LDA) in THF at -78 °C was used to generate the anion of (308). When the reaction was quenched with D_2O, no incorporation of the deuterium was detected as judged by ^1H NMR spectroscopy. Quenching the reaction with acetaldehyde, followed by aqueous work-up, only gave a complex mixture of products from which no product could be isolated. In an attempt to prevent these undesirable reactions, acetaldehyde was added to a 1:1 LDA-HMPA complex.[12] This complex is known to prevent the Michael addition reaction occurring at -78 °C. However, this too did not help, since a complex mixture of products was still obtained.

Another alternative was to convert (308) into the oxo- compound (309). Selenium dioxide is known to convert active methylene com-

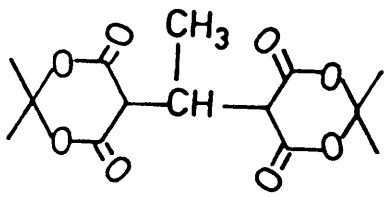


(309)

pounds of type (308) to ketones. Compound (309), if prepared, would be subjected to the Wittig reaction to give (307). However, reaction of (308) with selenium dioxide did not give any desired product, since only a complex mixture was obtained.

An interesting procedure, involving the reaction of Meldrum's acid with aldehydes and ketones catalysed by dry chromatographic neutral alumina to give compounds of type (307), has recently been reported.[13] In this procedure, reagents are simply absorbed on neutral alumina at room temperature without a solvent. However, acetaldehyde did not react with Meldrum's acid when we applied this procedure.

W. Lehuert[14] has reported the synthesis of a series of ethylidene malonates by a titanium tetrachloride/pyridine catalysed Knoevenagel condensation of malonic acid esters with aldehydes and ketones. We applied this procedure to Meldrum's acid (308) with acetaldehyde in dioxane at 0 °C for twenty-two hours in the presence of pyridine. Two main products were isolated. One was the desired product (307) and the other major component was the Michael addition product (310). Compound (307), although seriously contaminated by compound (310),



(310)

gave satisfactory spectral data. The IR spectrum contained absorption bands 1630 (C=C) and 1740 (C=O) [lit.,[14] 1632 (C=C) and 1738 cm^{-1} (C=O)]. The 60-MHz ^1H NMR spectrum in $CDCl_3$ indicated the olefinic proton resonating at δ 7.8 as a quartet. The mass spectrum gave a molecular ion at 170 (M^+).

Following the same procedure,[14] the unknown, *t*-butylcarbo-*t*-butyloxybut-2-enoate (306) was prepared in 28% yield from *t*-butyl malonate.[15] The IR spectrum gave the anticipated absorption bands at 1640 (C=C) and 1730 (C=O). The 60-MHz ^1H NMR spectrum in $CDCl_3$ showed a singlet at δ 1.5. corresponding to the 18H of the *t*-butyl group, a doublet at δ 1.75 for the vinyl methyl group and a quartet at δ 6.75 for the olefinic proton. The mass spectrum gave the expected ion at 130 [M-112]. The low yield of (306) is

probably due to hydrolysis of the product, to give acetaldehyde, malonic acid and isobutylene during aqueous work-up.

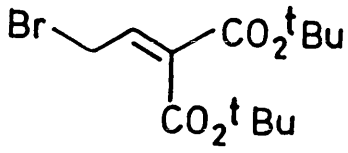
With compounds (306) and (307) in hand, the next step was to generate their anions with suitable bases and to react them with electrophiles. If alkylation takes place predominantly at the α- position, then silylation[16] to yield allyl silanes which, in the presence of a Lewis acid react with electrophiles to give γ-alkylation products, may be useful. The bulky groups close to the α- position in the ester might force alkylation to occur at the γ- position.

Thus, compound (306) was treated with LDA at -78 °C and then deuterated with D_2O. ^1H NMR spectrum did not indicate the presence of the deuterium. The mixture of LDA and (306) at -78 °C to room temperature with acetaldehyde, benzyl bromide and benzaldehyde and methyl iodide only gave complex mixtures of products after aqueous work-up. No desired products could be isolated from the mixture. Use of 1:1 LDA-HMPA complex before addition of electrophile did not change the outcome of the reaction. No starting material was recovered in any reaction. When aqueous work-up was avoided by directly absorbing the reaction mixture onto silica gel and subjecting to column chromatography, no desired product could be isolated at all.

It was not possible to generate the anion of (307) because the starting material was found to decompose during the process. The electrophile was always recovered quantitatively from the mixture. Meldrum's acid (308), too, was recovered. No further attempt was made to generate this anion.

The preparation of the Grignard and Reformatsky reagents from (306) and (307) was also investigated. Allylic bromination of (306)

with NBS gave the bromide (311) in quantitative yield. Compound (307) gave a complex mixture of products with a very low yield of the desired bromide.



(311)

Attempts to prepare the Reformatsky reagent from (311) were unsuccessful. Metallic zinc remained unreacted all the time and the starting material decomposed.

The Grignard reagent from (311) could not be prepared either, since the starting material decomposed in the process and only a complex mixture of products was obtained in each case. Because of other synthetic priorities, no further work was carried out on this line of research.

5.3 Preparation of Methyl 4-(1-*N*-benzyl-3-benzoyloxyazetidinyl)-2-trifluoroacetylaminobutanoate

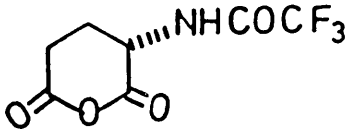
5.3.1 Introduction

As stated in the general introduction, compound (294b) was synthesised from *L*-glutamic acid by C. Smith.[7] We repeated the same procedure to prepare this compound, in order to investigate favourable conditions for its deprotection.

5.3.2 Results and Discussion

As reported by Ellis *et al.*,[8] treatment of *L*-glutamic acid with an excess of trifluoroacetic anhydride at 0 °C gave the anhydride

(312) in 55% yield, which was further refluxed in methanol to afford a 5:2 mixture of the α-ester (313a) and the γ-ester (313b) in 100% yield. Without further purification,



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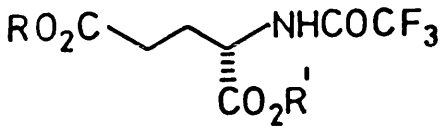
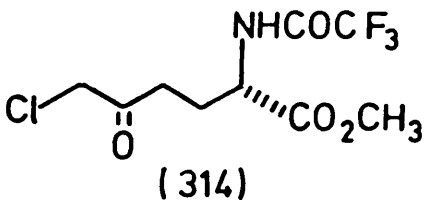


(313) (a) R = H, R' = CH_3

(b) R = CH_3, R' = H

this mixture was dissolved in ether and treated with 0.7 equivalents of dicyclohexylamine at 0 °C. The resulting precipitate was recrystallised from dioxane to give the pure dicyclohexylamine salt of (313a). Hydrolysis of this salt with 2 N hydrochloric acid gave, after extraction into ethyl acetate, the pure α-ester (313a) as a colourless viscous oil in 36% yield from (312). Exposure of the acid in (313a) to 1.5 equivalents of thionyl chloride in ether-DMF (100:1) at 0 °C gave the acid chloride as a white crystalline product which was found to be unstable at room temperature. It was therefore immediately treated with excess of diazomethane and subsequently with gaseous hydrogen chloride to yield the α-chloroketone (314) in 60% yield as a white crystalline product. Treatment of (314) with one



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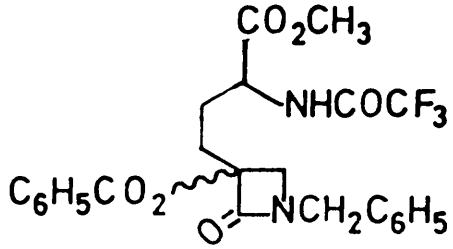
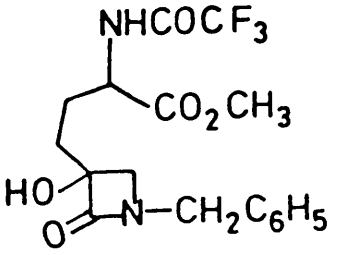


(294b)

equivalent each of benzyl isocyanide and benzoic acid without a solvent at room temperature for 15 hours gave the amide (293b) in 79% yield as a viscous yellow oil on standing. Cyclisation[9] of (293b) to (294b) was achieved by refluxing in THF with caesium fluoride in the presence of benzyltriethylammonium bromide and (294b) was isolated in 62% yield.

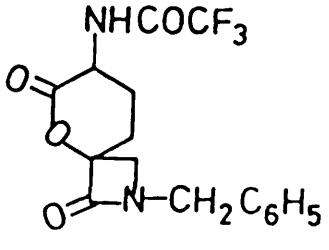
5.3.3 Attempted deprotection of Methyl 4(1-*N*-benzyl-3-benzoyloxyazetidinyl)-2-trifluoroacetylaminobutanoate

Much effort was put into the deprotection of (294b). From the initial studies,[7] it was found that the benzoyl group in (294b) could be cleaved with methanol/CH_3ONa at room temperature to give the tertiary alcohol (315). The yield of this alcohol was only 13% and the



(315)

product had to be isolated from a mixture of products. Following the same procedure, we could only improve the yield to 44%. We had originally anticipated that (315) would cyclise to the lactone (316) while in solution. However, this did not happen, so the



(316)

solution was acidified to pH 7 with 2 N hydrochloric acid. TLC analysis revealed that the sample was still unchanged until the pH was adjusted to pH 4 when a complex mixture of products resulted. No desired product could be isolated from this mixture.

Compound (315) was then subjected to hydrolysis with 10% aqueous sodium hydroxide[18] in methanol/water for one hour until TLC analysis indicated only a baseline spot. Close examination of this solid by ^1H NMR spectroscopy revealed that although hydrolysis had occurred, the product decomposed. TLC analysis of this solid using water/methanol on silica gel or alumina, revealed the presence of several spots. On acidification of the solid in water, more spots were revealed.

The same result was obtained when hydrolysis was conducted with KOH/CH_3OH-H_2O, K_2CO_3/CH_3OH and $LiOH/CH_3OH$. The reaction with $TMSiI/CH_3CN$[19] did not affect the ester since only starting material was recovered both for compound (315) and (294b).

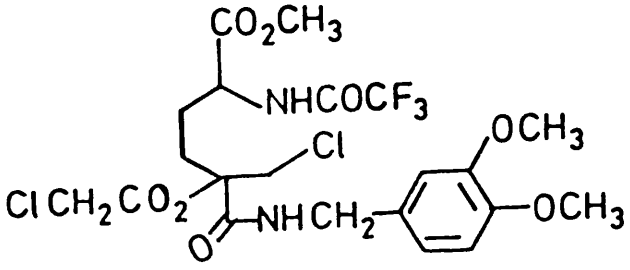
The benzyl group proved too difficult to cleave. Catalytic hydrogenation on Pd/C, $PdCl_2$ or PtO_2 did not remove the benzyl group at all. In the case of PtO_2, a complex mixture of products resulted when the hydrogen pressure was increased to 100 psi. Catalytic transfer hydrogenation either with 4.4% formic acid on palladium black,[20] or with ammonium formate[21] on palladium on carbon did not give any reaction at all.

It became obvious that the two protecting groups, one on the ring nitrogen and the other on the hydroxyl group at C_3 in the ring, had to be changed to more easily cleaved ones. Selective cleavage of the ring nitrogen protecting group would probably facilitate the removal of the remaining groups at least under basic conditions.

The following section describes the efforts made in this line.

5.4 Preparation of Methyl-5-chloroacetoxy-6-chloro-5(3,4-dimethoxybenzyl)carbamoyl-2-trifluoroacetylaminohexanoate

Precursor (318) was prepared from (314) by reaction with chloroacetic acid and 3,4-dimethoxybenzyl isocyanide (the Passerini reaction).[9] We anticipated that the chloroacetyl group would easily be cleaved with thiourea to give a higher yield of the tertiary alcohol. The dimethoxybenzyl group should also be easily cleaved.



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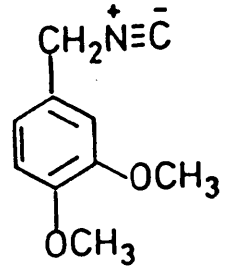


(317)

3,4-Dimethoxybenzyl isocyanide (317) is not commercially available. Although it could easily be prepared from 3,4-dimethoxybenzylamine by the method of Weber and Gokel,[22] the best yield was only 42%. The product was found to be very unstable on standing, since it quickly darkens, breaking into several components. It was therefore used immediately after isolation. Attempts were made to improve the yield of this product. Thus, 3,4-dimethoxybenzylamine was converted to the corresponding 3,4-dimethoxybenzylformamide by treatment with formic acid in refluxing toluene for 3 days. Attempts to dehydrate this formamide to give the isocyanide were unsuccessful. Phosphoryl chloride[23] in dichloromethane in the presence of di-

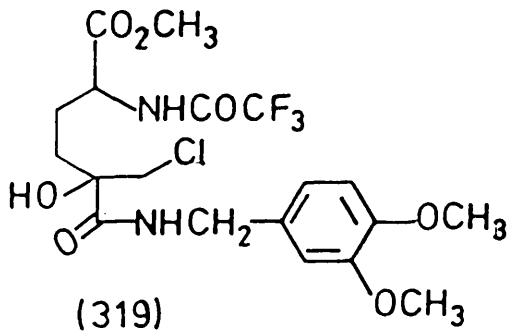
isopropylamine gave no reaction. *p*-Toluenesulphonyl chloride[24] in pyridine also gave no reaction.

The preparation of (318) had to be conducted in dichloromethane, as a mixture of chloroacetic acid, the ketone (314) and 3,4-dimethoxybenzyl isocyanide was too viscous to stir and even to mix. The reaction could be conducted at room temperature for 3 days. When heated under reflux, the reaction was complete after twenty hours. It was isolated in 52% as a white crystalline product, which gave satisfactory elemental analysis. The IR spectrum showed absorption bands at 3420 (NH), 1730-1750 (C=O esters), 1680 (NHC=O) and 1600 cm^{-1} (aromatic). The 60-MHz ^{1}H and ^{13}C NMR spectra were consistent with the structure. The mass spectrum gave the expected molecular ions at 561 (M+1) and 525 (M-HCl).

5.4.1 Attempted Cyclisation of Methyl 5-chloroacetoxy-6-chloro-5(3,4-dimethoxybenzyl)carbamoyl-2-trifluoroacetylaminohexanoate and Attempted Deprotection

Cyclisation of the chloroamide (318) was not achieved at all. Caesium fluoride both at room and refluxing temperature in THF in the presence of benzyltriethylammonium bromide only gave complex mixtures of products. Sodium hydride both in DMF-dichloromethane (1:1), 80 °C, 5 hours, and in THF, -78 °C, room temperature,[25] also only gave complex mixtures of products. Pyridine in both refluxing and cold ethanol only gave decomposition products. Potassium hydroxide[26] absorbed on neutral alumina was also reacted with (318) in hot dioxane and gave hydrolysis products, from which no desired product was isolated.

It was obvious that the chloroacetyl group probably took part in all the reactions resulting in complex mixtures of products. Its removal was therefore investigated. Thus, the chloroacetyl ester (318) was treated with thiourea[27] in pyridine in the presence of ethanol for 2 hours under reflux and gave a mixture of products from which a small amount of product was isolated by column chromatography. The IR spectrum showed an additional absorption band at 3450 cm^{-1} (OH), together with the NH absorption band at 3400 cm^{-1}. The ^1H NMR spectrum no longer indicated the presence of the chloroacetyl protons at 4.05 ppm. The mass spectrum gave the expected molecular ion at 484 (M), corresponding to methyl 6-chloro-5-(3,4-dimethoxybenzyl)carbamoyl-5-hydroxy-2-trifluoroacetylamino hexanoate (319). The sample was found to be unstable on standing and decomposed into



(319)

several components. The same result was obtained when (318) was treated with one equivalent of a weak base such as sodium acetate in methanol-water. Treatment of (318) with NaOH and LiOH in methanol only gave hydrolysis products which, on acidification, gave a complex mixture of products.

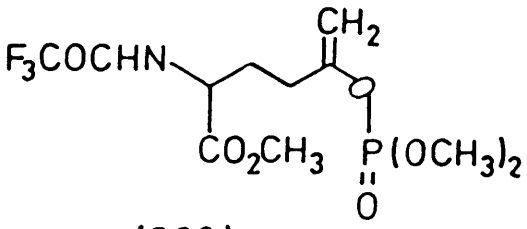
The cleavage of 3,4-dimethoxybenzyl group was also investigated. Catalytic hydrogenation, Pd/C, H_2, gave no reaction. Potassium persulphate/$Na_2HPO_4.2H_2O$-aqueous CH_3CN[28] only gave a complex mixture

of products. It appeared, therefore, that deprotection of either (318) or the β-lactam (294b) cannot be achieved, since only decomposition products result. The β-lactam ring is affected by both basic and acidic hydrolysis.

5.5 Preparation of (S)-Methyl 6-dimethoxyphosphoryl-5-oxo-2-trifluoroacetylaminohexanoate

5.5.1 Introduction

The reaction of either the acid chloride (292b) or the α-chloroketone (314) with phosphorus reagents was also investigated. The α-chloroketone (314) should react with trimethyl phosphite to give either the vinyl phosphate (320) *via* the Perkov reaction, or the phosphonate (321) *via* the Arbusov reaction.[29] Subsequent hydrolysis of these proucts could give possible inhibitors of GS. Compound (321)



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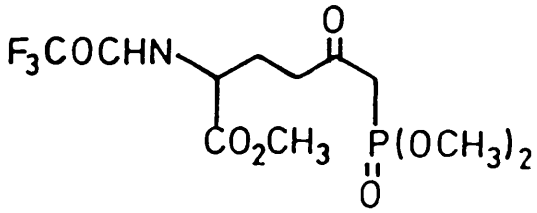


(321)

should also be available from the acid chloride (292b) with dimethyl methyl phosphonate under base conditions. The reactions of (292)

and (314) with these reagents are discussed.

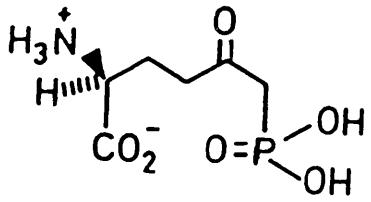
5.5.2 Results and Discussion

Trimethyl phosphite failed to react with one equivalent of the α-chloroketone (314) in refluxing toluene even after two days. Excess of trimethyl phosphite did not change the outcome of the reaction. The ketone was recovered in each case.

Heating the α-chloroketone (314) in neat trimethyl phosphite gave a mixture consisting of several components after five hours. Chromatographic separation of the mixture on silica gel gave a small amount of colourless oil. The IR spectrum of this oil showed absorption bands at 1660 (C=O, amide), 1730 (C=O, ester), 1750 (C=O, ketone). The 100-MHz ^1H NMR spectrum in $CDCl_3$ indicated a 6H doublet corresponding to the two methoxy groups attached to phosphorus and appeared at 3.79 ppm. The methoxyl protons of the ester group appeared at 3.84 ppm. The methylene protons next to the phosphoryl group appeared at 4.60 ppm. The methine proton gave a multiplet at 4.80 ppm. The NH proton appeared at 8.05 ppm. There were no olefinic protons in the spectrum. The mass spectrum gave a molecular ion at m/z FAB(+) 364 (M+1) and 362 (M-1), FAB(-), 285 $[M-(CH_3O)_2\overset{+}{P}H]$, corresponding to (S)-methyl 6-dimethoxyphosphoryl-5-oxo-2-trifluoroacetylamino hexanoate (321).

Treatment of the acid chloride (292) with an enolate ion generated from dimethyl methyl phosphonate with LDA in THF gave a complex mixture of products. Chromatographic treatment of this mixture gave a trace amount of oil which gave a molecular ion at m/z 364 (M+1) in the mass spectrum. The IR spectrum also showed absorption bands at 1730-1750 (C=O), 1670 (C=O, amide) and 1270 (P=O). The data seem to correspond

to the phosphonate (321). It appeared, therefore, that the product is formed *via* the Arbusov reaction. Under appropriate conditions (321) should give (322), which is a possible inhibitor of glutamine



(322)

synthetase. Due to lack of time, deprotection of (321) was not undertaken. Thus the S-isomer of (321) is available from S-glutamic acid.

EXPERIMENTAL

EXPERIMENTAL

Melting points were determined using an electrothermal melting point apparatus and are uncorrected. Optical activity was recorded on a Perkin-Elmer 141 polarimeter. Infra-red spectra were recorded on a Perkin-Elmer IR spectrophotometer 197 and Perkin-Elmer 1310 IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 60 MHz with a Varian anaspect EM 360 NMR spectrometer, Hitachi Perkin-Elmer HR NMR spectrometer R-24B, at a 100 MHz on a JEOL JNM-PS-100 FT NMR spectrometer and at 270 MHz on a JEOL JNM-GX270 FT NMR spectrometer. Mass spectra were recorded on a VG analytical 7070E spectrometer.

Reactions were monitored by T.L.C. on Merck Kieselgel 60 PF_{254} or Merck aluminiumoxid 60 PF_{254} neutral (type E) using an appropriate eluant. Column chromatography was carried out using pressurised short-path columns with either silica gel type 60H Merck No.7736 or aluminium oxide 60 PF_{254} neutral (type E) or using pressurised sintered filter funnels. Detection of products was carried out using:-

(a) ultraviolet irradiation of T.L.C. Plates impregnated with a fluorescent indicator;

(b) iodine vapour used as a general, but indiscriminating, reagent;

(c) development using a 5% ethanolic solution of anisaldehyde with a trace amount of acetic acid and concentrated sulphuric acid and heating was often used. With this reagent each reaction component generally developed at a different rate to afford a different colour. Hence coincidental components or compounds, with similar R_f values could be distinguished;

(d) development using ninhydrin reagent was useful in detecting the amino compounds of the mixture;

(e) development using 20% ethanolic solution of phosphomolybdic acid (PMA) was found to be extremely sensitive, but indiscrimanating.

Solvents

Petroleum ether refers to the fraction of b.p. 60-80 °C and ether refers to diethyl ether. Other solvents were reagents grade unless otherwise stated. Solvents used for chromatography were redistilled and reaction solvents were generally dried by an appropriate procedure, redistilled and stored under nitrogen. In particular, tetrahydrofuran was pre-dried over sodium wire, then refluxed over sodium benzophenone ketyl until dry and redistilled immediately prior to use.

Nitrogen refers to dry oxygen-free nitrogen.

Preparation of (S)-(+)-5-Carboxybutyrolactone (180)

This compound was prepared according to the procedure of Yamada *et al.* as described below.[1,4]

To a suspension of S-glutamic acid (178) (90 g, 0.612 mol) in water (240 ml) and concentrated hydrochloric acid (126 ml) was added a solution of sodium nitrite (63 g, 0.912 mol) in water (135 ml) during 4 h under vigorous stirring at 0 °C. The resultant clear solution was allowed to warm to room temperature overnight. The solvent was evaporated *in vacuo* to dryness below 50 °C to give a residue which was shaken with ethyl acetate (300 ml). The insoluble inorganic salts were filtered off through a pad of celite and washed with ethyl acetate. The filtrate and washings were dried over sodium sulphate. Evaporation of the solvent *in vacuo* afforded (S)-(+)-5-carboxybutyrolactone (180) (69.62 g) as a pale yellow syrup. ν_{max} (neat film) 3500-3000 (OH, br), 1770 (C=O, lactone) and 1730 cm^{-1} (C=O, acid), m/z 130 ($\underline{M}^+$).

Preparation of (R)-(-)-5-Carboxybutyrolactone

This compound was prepared in the same way as the S-isomer from R-glutamic acid (179).[4]

Preparation of (S)-(+)-5-Ethoxycarbonylbutyrolactone (182)

(S)-(+)-5-Carboxybutyrolactone (180) (69.62 g) and *p*-toluenesulfonic acid (2 g) in dry ethanol (130 ml) and dry benzene (300 ml) were heated under reflux for 5 h. The solvent was evaporated *in vacuo*. Benzene (200 ml) was added to the residue. Water (100 ml) was added to the mixture, partitioned and the organic layer was further washed with 10% sodium carbonate (100 ml) and dried(Na_2SO_4). Evaporation of the solvent *in vacuo* and distillation of the residue at reduced pressure gave a colourless oil (59.48 g). ν_{max} (thin film) 1730 (C=O, ester) and 1770 cm^{-1} (C=O, lactone), m/z 158 (M^+).

Preparation of (S)-(+)-5-Hydroxymethylbutyrolactone (181)

Method 1 This compound was prepared according to the procedure of Taniguchi *et al.* as described below.[1]

To a stirred suspension of sodium borohydride (1.6 g, 0.04 mol) in ethanol (28.3 ml) was added a solution of (182) (10.0 g, 0.06 mol) in ethanol (45.5 ml) at room temperature. After 1 h, the mixture was acidified to pH 3 with 2 N hydrochloric acid and the precipitate formed was filtered off. The filtrate was evaporated *in vacuo*. Methanol (50 ml) was added and evaporated. This was repeated four times. The residue was finally purified by column chromatography (silica gel) to afford a colourless oil (3.03 g, 41%). ν_{max} (thin film) 3400 (OH, br) and 1770 cm^{-1} (C=O), m/z 116 (M^+).

Method 2[4] To a stirred solution of (180) (10.8 g, 83 mmol) in THF (75 ml) under a nitrogen atmosphere at room temperature was added borane-methyl sulphide-T.H.F. complex (48.45 ml) over 50 min. After

stirring for 3 more h, the mixture was cautiously quenched by addition of dry methanol (60 ml). The solvent was evaporated *in vacuo* and more methanol (3 x 100 ml) was added and evaporated. The residue was then distilled at reduced pressure to give 7.26 g (75%) of pale yellow oil which gave satisfactory spectral data.

Preparation of (R)-(-)-5-Hydroxymethylbutyrolactone

Like the S-isomer, this compound was similarly prepared according to the procedure of Silverstein *et al.*[4] as described above in Method 2 and was prepared in 78% yield.

Preparation of (S)-(+)-5-(*p*-toluenesulphonyloxymethyl)butyrolactone (183)

This compound was prepared according to the method of Mori[3] and modified by Olsen *et al.*[5] as described below.

The alcohol (181) (8.92 g, 0.08 mol) in dry pyridine (64 ml) at 0 °C was stirred with *p*-toluenesulphonyl chloride (24.88 g, 0.13 mol) and 4-dimethylaminopyridine (0.93 g, 7.62 mmol) for 24 h, poured into ice-cold 2N hydrochloric acid (100 ml) and extracted with ethyl acetate (3 x 100 ml). The organic layer was washed with water (100 ml), brine (50 ml) and dried ($MgSO_4$) to give 17 g of yellow oil. Crystallisation from ethyl acetate-petroleum ether gave 12.0 g (57%) of white crystals, m.p. 85-86 °C. ν_{max} ($CHCl_3$) 1770 (C=O, lactone), 1600 (aromatic) and 1365 cm^{-1} (S=O), m/z 270 ($\underline{M}^+$).

Preparation of (R)-(-)-5-(*p*-toluenesulphonyloxymethyl)butyrolactone

Like the S-isomer, this compound was prepared according to the modified procedure of Olsen *et al.*[5] and was isolated in 67% yield. It gave satisfactory spectral data.

Preparation of (S)-(+)-5-Azidomethylbutyrolactone (184)

This compound was prepared according to the procedure in the reference as described below.[5]

To a stirred solution of (183) (1.02 g, 3.78 mmol) in *N,N*-dimethylformamide (15 ml) at room temperature under a nitrogen atmosphere was added sodium azide (1.47 g, 22.61 mmol) and the mixture was refluxed for 1 h, cooled and the solvent was evaporated *in vacuo*. The residual solid was extracted with chloroform (100 ml) and filtered to give, after evaporation of the solvent at reduced pressure, a crude yellow oil which was purified by dry-column flash chromatography (silica gel) eluting with ethyl acetate-petroleum ether to afford 0.46 g (87%) of colourless oil. ν_{max} (thin film) 2100 (azido) and 1770 cm^{-1} (C=O), δ_H (100 MHz; $CDCl_3$) 1.82-2.68 (4H, m, $CHC\underline{H}_2C\underline{H}_2CO_2$), 3.32-3.72 (2H, m, $N_3C\underline{H}_2CH$) and 4.56-4.80 (1H, m, $N_3CH_2C\underline{H}CH_2$), δ_C (100 MHz; $CDCl_3$) 176.61 (C1), 28.22 (C2), 24.49 (C3), 78.34 (C4) and 54.17 (C5), m/z 141 ($\underline{M}^+$) and 85 (100 %).

Preparation of (R)-(-)-5-Azidomethylbutyrolactone (188)

The preparation of this compound was analogous to that of the S-isomer and was isolated in 99% yield. ν_{max} (thin film) 2100 (azido) and 1770 cm^{-1} (C=O), δ_H (100 MHz; $CDCl_3$) 1.82-2.68 (4H, m, $C\underline{H}_2C\underline{H}_2CO$), 3.26-3.80 (2H, m, $N_3C\underline{H}_2OH$) and 4.48-4.84 (1H, m, $N_3CH_2C\underline{H}CH_2$), δ_C (100 MHz; $CDCl_3$) 176.72 (C1), 28.22 (C2), 24.59 (C3), 78.06 (C4) and 54.23 (C5), m/z 142 (M+1), 114 ($M-N_2$) and 85.

Preparation of (S)-(+)-5-Aminomethylbutyrolactone Hydrobromide (186)

To a solution of (S)-(+)-5-azidomethylbutyrolactone (184) (0.32 g, 2.27 mmol) in dry methanol (5 ml) was added 10% palladium on carbon (80 mg) and 48% hydrogen bromide (1 ml). The mixture was stirred for 15 h under a hydrogen atmosphere at room temperature. The catalyst

was filtered off and the filtrate was evaporated at reduced pressure to leave a colourless gum which was triturated with dry acetone to give 0.41 g (94%) of white crystals. $[\alpha]_D^{23}$ 51.43 (C 1.55, H_2O), ν_{max} (Nujol) 1750 (C=O) and 1560 cm^{-1}($\overset{+}{N}H_3$), δ_H (270 MHz; D_2O) 1.82-2.24 (2H, m, $C\underline{H}_2CH_2CO$), 2.40-2.80 (2H, m, $CHCH_2C\underline{H}_2CO$) and 3.0-3.44 (3H, m, $N\underline{C}H_2\underline{C}H\text{-}CH_2$), δ_C (270 MHz; D_2O) 182.03 (C1), 29.42 (C2), 25.73 (C3), 79.09 (C4) and 44.21 (C5) [Found: C, 30.98; H, 5.35; N, 7.51 m/z, 115 (M-HBr). $C_5H_{10}O_2NBr$ requires C, 30.63; H, 5.14; N, 7.14%, M, 196.0490].

Preparation of (R)-(-)-5-Aminomethylbutyrolactone Hydrobromide (187)

As in the preparation of the S-isomer, this compound was prepared from (R)-(-)-5-azidomethylbutyrolactone (188) and was isolated in 86% yield. $[\alpha]_D^{23}$ -51.43 (C 1.55, H_2O), ν_{max} (Nujol) 1750 (C=O) and 1560 cm^{-1} ($\overset{+}{H_3N}$); δ_H (270 MHz; D_2O) 1.82-2.24 (2H, m, $C\underline{H}_2CH_2CO$), 2.62 (2H, m, $CH_2C\underline{H}_2CO$) and 3.0-3.44 (3H, m, $\overset{+}{H_3N}C\underline{H}_2C\underline{H}CH_2$), δ_C (270 MHz; D_2O) 181.92 (C1), 29.36 (C2), 25.68 (C3), 79.04 (C4), and 44.10 (C5). [Found: C, 30.35; H, 5.17; N, 7.14%; m/z, 115 (M-HBr). $C_5H_{10}O_2NBr$ requires C, 30.63; H, 5.14; N, 7.14%; M, 196.0490].

Prepration of (±)-Diethyl formylsuccinate (190)

This compound was prepared according to the procedure of Tocanne as described below.[9]

Diethyl succinate (19.9 g, 0.11 mol), ethyl formate (11.92 g, 0.16 mol) and sodium metal (3 g, 0.13 mol) were stirred together in ether (100 ml) for 20 h at 0 °C. The reaction mixture was diluted with water (100 ml) and partitioned. The aqueous layer was acidified with 2N sulphuric acid and extracted with ether (2 x 50 ml). The extracts were dried (Na_2SO_4) and evaporated *in vacuo* to give 18.57 g (80%) of yellow oil.

Preparation of Diethyl Hydroxymethylsuccinate (191)

This compound was prepared according to the modified standard procedure as decribed below.[9]

To a stirred diethyl formylsuccinate (190) (5.0 g, 0.025 mol) in methanol (50 ml) at 0 °C was added sodium borohydride (1.03 g, 0.027 mol) in portions. After stirring for 2 h, the reaction was poured onto ice and was extracted with ether (3 x 50 ml). The organic layer was dried ($MgSO_4$) and evaporated to give 4.14 g (82%) of pale green oil.

Preparation of (±)-Paraconic Acid (189)

This compound was prepared according to Mori's procedure as described below.[10]

Diethyl hydroxymethylsuccinate (191) (3.66 g, 0.018 mol) was dissolved in 95% ethanol (5 ml). Potassium hydroxide (2.4 g, 0.043 mol) in water (22 ml) was added in portions and the mixture was heated under reflux for 1 h, cooled to room temperature and then filtered through a column of ion-exchange resin, Amberlite IR-120 in the H^+-form, and was eluted with water. The eluant was immediately evaporated *in vacuo* below 50 °C to give a pale yellow oil (2.27 g, 97%), which crystallised on standing, m.p. 59-60 °C.

Preparation of (±)-4-Hydroxymethylbutyrolactone (192a)

This compound was prepardd according to the literature procedure.[10] To a stirred solution of paraconic acid (189) (3.81 g, 0.029 mol) in T.H.F. (26 mol) under a nitrogen atmosphere at 0 °C was added borane-methyl sulphide complex in T.H.F. (18.17 ml) *via* a pressure-equalising dropping funnel over 15 min. After stirring for 3 h, dry methanol (25 ml) was cautiously added to destroy the excess borane complex. The solution was concentrated *in vacuo* and the residue was redissolved

in methanol (25 ml) and evaporated *in vacuo*. This was repeated once more. The residue was finally purified by dry-column flash chromatography on silica gel using methanol-dichloromethane (1:7 v/v) as eluant to afford 2.89 g (85%) of colourless oil. ν_{max} ($CHCl_3$) 3450 (OH, br) and 1770 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 2.38 (1H, d of d, J = 5.7 Hz and J_{AB} = 17.60 Hz, $C\underline{H}_aH_bCO$), 2.62 (1H, d of d, J = 8.80 Hz and J_{AB} = 17.60 Hz, $CH_a\underline{H}_bCO$), 2.75 (1H, m, $C\underline{H}$), 4.22 (2H, m, $\underline{HOCH}_x\underline{H}_yCH$), 4.43 (1H, d of d, J = 7.33 Hz and J = 9.16 Hz, $HOCH_x\underline{H}_yCH$) and 3.58-3.80 (2H, m, $C\underline{H}_2OCO$), δ_C (270 MHz; $CDCl_3$) 178.65 (30%, $\underline{C}$=O), 30.96 (100%, $C\underline{H}_2CO$), 37.01 (91% $\underline{C}H$), 71.16 (74%, $C\underline{H}_2OCO$) and 62.75 (95%, $HOC\underline{H}_2CH$).

Preparation of (±)-4-(*p*-Toluenesulphonyloxymethyl)butyrolactone (192b)

(192a) (1.41 g, 0.01 mol), Tosyl chloride (3.95 g, 0.25 mol) and *N,N*-dimethylaminopyridine (0.15 g, 1.23 mmol) were stirred in dry pyridine (14 ml) at 0 °C for 48 h, diluted with 2N hydrochloric acid (20 ml) and extracted with ethyl acetate (3 x 50 ml). The combined organic layer was washed with water (50 ml), brine (50 ml) and dried ($MgSO_4$). The solvent was removed at reduced pressure to give 2.6 g of colourless viscous oil, a crude product which was subjected to dry-column flash chromatography on silica gel using ethyl acetate-petroleum ether as eluant to afford (±)-4-(*p*-toluenesulphonyloxymethyl)butyrolactone (192b) (2.44 g, 74%) as a colourless viscous oil on standing. ν_{max} ($CHCl_3$) 1590 (aromatic) and 1770 cm^{-1} (C=O, lactone), δ_H (270 MHz; $CDCl_3$) 2.28 (1H, d of d, J = 6.41 Hz and J_{AB} = 17.81 Hz, $C\underline{H}_aH_bCO$), 2.46 (3H, s, $C\underline{H}_3C_6H_4$), 2.63 (1H, d of d, J = 9.07 Hz and J_{AB} = 17.81 Hz, $CH_a\underline{H}_bCO$), 4.04 (1H, m, $C\underline{H}$), 4.07 (3H, m, $SO_2OC\underline{H}_2CH$ and $CHC\underline{H}_x\underline{H}_yOCO$), 4.38 (1H, d of d, J = 7.69 Hz and J = 9.61 Hz, $CHH_x\underline{H}_yOCO$), 7.39 (2H, d, J_{AB} = 8.15 Hz, aromatic) and 7.79 (2H, d, J_{AB} = 8.15 Hz, aromatic) δ_C (270 MHz; $CDCl_3$) 175.49 (10%, C=O), 30.47 (42%, C2), 34.63 (48%, C3),

69.43 (58%, C4), 69.43 ($SO_2O\underline{C}H_2CH$), 145.55 (20%), 132.19, 130.18, 130.10, 130.06 and 127.89 (aromatics). [Found: $\underline{M}^+$, 270.0539. $C_{12}H_{14}O_5S$ requires $\underline{M}$, 270.0560].

Preparation of (±)-4-Azidomethylbutyrolactone (192c)

(192b) (1.01 g, 3.74 mmol) and sodium azide (1.45 g, 22.31 mmol) were heated under reflux in *N,N*-dimethylformamide (75 ml) for 1 h. The solvent was evaporated *in vacuo* and the residue was triturated with chloroform, filtered through a pad of celite and the filtrate and washings were concentrated *in vacuo* to leave 0.68 g of crude yellow oil which was purified by column chromatography (silica gel) eluting with ethyl acetate-petroleum ether to give (±)-4-azidomethylbutyrolactone (192c) (0.41 g, 77%) as a pale yellow oil. ν_{max} ($CHCl_3$) 2100 (azido) and 1770 cm^{-1} (C=O, lactone), δ_H (270 MHz; $CDCl_3$) 2.36 (1H, d of d, J = 6.23 Hz and J = 17.58 Hz, $C\underline{H}_aH_bCO$), 2.67 (1H, d of d, J = 8.79 Hz and J = 17.58 Hz, $CH_a\underline{H}_bCO$), 2.81 (1H, m, $\underline{C}H$), 3.49 (2H, m, $N_3C\underline{H}_2CH$), 4.12 (1H, d of d, J = 9.48 Hz and J = 5.59 Hz, $C\underline{H}_xH_yOCO$) and 4.42 (1H, d of d, J = 7.33 Hz and J = 9.48 Hz, $CH_x\underline{H}_yOCO$), δ_C (270 MHz; $CDCl_3$) 176.04 (15%m C1), 31.66 (10%, C2), 35.03 (75%, C3), 70.48 (78%, C4) and 52.79 (96%, $N_3C\underline{H}_2$), m/z 142 (M+1), 116 and 85 (100%).

Preparation of (±)-4-Aminomethylbutyrolactone Hydrobromide (175)

(192c) (0.29 g, 2.06 mmol), 10% palladium on carbon (80 mg) and 48% aqueous hydrogen bromide (1 ml) were stirred in methanol (5 ml) at room temperature for 16 h under a hydrogen pressure. The catalyst was filtered through celite and the filtrate was evaporated *in vacuo*. The residue was taken up in water (5 ml) and extracted with chloroform (3 x 5 ml), the aqueous layer was evaporated *in vacuo* to dryness, and the residue was redissolved in methanol (5 ml) and heated under reflux

for 5 min with activated charcoal, filtered through a pad of celite to leave, after evaporation of the solvent, a colourless hygroscopic gum (0.4 g, 100%), which could not be crystallised. ν_{max} (Nujol) 1600 ($\overset{+}{N}H_3$) and 1760 cm^{-1} (C=O, lactone), δ_H (270 MHz; D_2O) 1.81 (1H, m, CH_aH_bCO), 2.14 (1H, m, CH_aH_bCO), 2.46 (2H, m, $H_3\overset{+}{N}CH_xH_yCH$), 2.91 (1H, m, $H_3\overset{+}{N}CH_xH_yCH$), 3.46 (1H, m, $CHCH_aH_bOCO$) and 3.86 (1H, m, $CHCH_aH_bOCO$), δ_C (270 MHz; D_2O) 179.90 (45%, C1), 32.32 (54%, C2), 34.89 (18%, C3), 71.57 (70%, C4) and 41.10 (53%, $H_3\overset{+}{N}CH_2$), m/z 115 (M^+-HBr), 80 (10%, HBr). Due to the hygroscopic nature of this compound, satisfactory elemental analysis could not be obtained.

Preparation of 4-Iodomethyloxetan-2-one (195)[14]

But-3-enoic acid (5 g, 58.1 mmol) was added to a suspension of freshly prepared silver isocyanate (15.25 g, 101.67 mmol) at 0 °C in acetonitrile (100 ml). Iodine (19.46 g, 76.61 mmol) was added to the mixture. The whole was stirred at 0 °C under a nitrogen atmosphere for 6 h. The inorganic salts were filtered off through a pad of celite and the filtrate was concentrated *in vacuo* to a small volume. This was diluted with water (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic layer was washed with a saturated solution of sodium bicarbonate (2 x 100 ml), with 10% sodium thiosulphate (100 ml) and water (50 ml). After drying (Na_2SO_4) filtration through a pad of alumina afforded 4-iodomethyloxetan-2-one (195) (6.82 g, 55%) as a colourless oil which gradually darkens on standing in the light. ν_{max} (thin film) 1825 cm^{-1} (C=O), m/z 212 (M^+), 127 (I), 85 (100%).

Preparation of 4-Azidomethyloxetan-2-one (196)

This compound was prepared according to the method of Scowen as described below.[19]

(195) (3 g, 14.2 mmol) was dissolved in dimethylsulphoxide (120 ml) under an atmosphere of nitrogen. Sodium azide (0.92 g, 14.2 mmol) and silver tetrafluoroborate (2.76 g, 14.2 mmol) were added each in one portion. The mixture was then stirred for 6 days at room temperature, poured into water(500 ml) and extracted alternatively with dichloromethane (4 x 125 ml) and ether (3 x 125 ml) to give, after drying (Na_2SO_4) and evaporation of the solvent *in vacuo*, 5.6g of yellow oil. This oil contained a lot of dimethylsulphoxide. The product was isolated by column chromatography (silica gel) to afford 0.5 g (26%) of pale yellow oil, ν_{max} (thin film) 1830 (C=O), 1110 (C-O) and 2100 cm^{-1} (azido), m/z 71 (base), 127 (M^+).

Attempted Preparation of 4-Aminomethyloxetan-2-one Hydrobromde (197)

4-Azidomethyloxetan-2-one (196) (0.2 g, 1.57 mmol) was dissolved in dry methanol (3 ml) under a nitrogen atmosphere. 48% hydrogen bromide (0.5 ml) and 0.05 g of 10% palladium on carbon were added, each in one portion. The mixture was flushed with hydrogen and was then stirred under a pressure of hydrogen for 3 days. The solvent was evaporated at reduced pressure, after filtration of the catalyst, to leave a colourless oil which was found to be a complex mixture of products by TLC analysis. The IR analysis of this mixture showed the absence of both the azido and the lactone carbonyl absorption bands.

Preparation of (S)-(+)-Sodium-5-azido-4-hydroxypentanoate (203)

To a stirred solution of (S)-(+)-4-azidomethylbutyrolactone (184) (0.4 g, 2.84 mmol) in dry methanol (5 ml) was added sodium hydroxide (0.1141 g, 2.84 mmol). The mixture was heated under reflux for 2½ h. The solvent was evaporated *in vacuo*. The residue was redissolved in water (3 ml) and extracted with ethyl acetate (3 x 5 ml). The aqueous layer was freeze-dried to leave hygroscopic white crystals (0.4326 g, 92%).

ν_{max} (Nujol) 3200-3460 (br OH), 2100 (azido) and 1550 cm^{-1} (CO_2Na), δ_H (270 MHz; CD_3OD) 1.67-1.90 (2H, m, $\underline{CH_2}CH_2CO_2Na$), 2.30 (2H, t, J = 6.5 Hz, $CH_2\underline{CH_2}CO_2$), 3.17-3.30 (2H, m, $N_3\underline{CH_2}CH$) and 3.70-3.79 [1H, m, $N_3CH_2\underline{CH}(OH)CH_2$], δ_C (270 MHz; CD_3OD) 182.43 (C1), 31.95 (C3), 35.29 (C2), 57.78 (C5) and 72.05 (C4), m/z, 180 (-) FAB (M-1), 158 (M-Na).

Preparation of (R)-(-)-Sodium-5-azido-4-hydroxypentanoate (204)

The preparation of this compound was analogous to that for the S-isomer and proceeded in quantitative yield. ν_{max} (Nujol) 3460 (br OH), 2100 (azido) and 1550 cm^{-1} (CO_2Na), δ_H (270 MHz; CD_3OD) 1.57-1.80 (2H, m, $\underline{CH_2}CH_2CO_2Na$), 2.20 (2H, J = 7.15 Hz, $CH_2\underline{CH_2}CO_2Na$), 3.07-3.17 [2H, m, $N_3\underline{CH_2}CH(OH)$] and 3.60-3.69 (1H, m, $\underline{CH}$), δ_C (270 MHz; CD_3OD) 182.89 (C1), 35.80 (C2), 32.46 (C3), 72.56 (C4) and 58.29 (C5), m/z 180 (-) FAB, 158 (M-Na).

Preparation of (S)-(+)-Sodium-5-amino-4-hydroxypentanoate (214)

A solution of (203) (0.52 g, 2.87 mmol) in methanol was hydrogenated at room temperature over 10% palladium on carbon (100 mg) for 22 h. The catalyst was filtered through a pad of celite. The filtrate was evaporated *in vacuo* to leave a colourless gum which was redissolved in water and extracted with chloroform (2 x 5 ml). The aqueous layer was evaporated *in vacuo* to leave 0.3 g (68%) of colourless hygroscopic gum. ν_{max} (Nujol) 3360 (br, OH, NH) and 1565 cm^{-1} (CO_2Na). δ_H (270 MHz; D_2O) 1.57-1.88 (2H, m, $\underline{CH_2}CH_2CO$), 2.14-2.34 (2H, m, $CH_2\underline{CH_2}CO$), 2.52-2.73 [2H, d of d, J_{AB} = 13.38 H and J_{AX} = 3.84 Hz, $NC\underline{H_2}CH(OH)$] and 3.53-3.62 (1H, m, $NCH_2\underline{CH}CH_2$), δ_C (270 MHz, D_2O) 182.95 (C1), 33.63 (C2), 30.47 (C3), 71.75 (C5) and 47.34 (C5), m/z 154 (-) FAB, 132 (M-Na).

Preparation of (R)-Sodium-5-amino-4-hydroxypentanoate (215)

As for the S-isomer, this compound was prepared from (204) in high yield. ν_{max} (Nujol) 3340 (br, OH, NH) and 1550 cm^{-1} (CO_2Na), δ_H (270 MHz; D_2O) 0.85-1.00 (2H, m, $C\underline{H}_2CH_2CO_2Na$), 1.46-1.55 (2H, m, $CH_2C\underline{H}_2CO_2Na$) 1.75-1.95 [2H, d of d, J_{AB} = 13.37 Hz, J_{AX} = 4.03 Hz, $H_2NC\underline{H}_2CH(OH]$ and 2.58-2.84 [1H, m, $H_2NCH_2C\underline{H}(OH)]$, δ_C (270 MHz; D_2O) 182.93 (C1), 33.72 (C2), 30.49 (C3), 72.23 (C4) and 48.81 (C5), m/z 154 (-) FAB, 132 (M-Na).

Preparation of (R)-Ethyl-5-azido-4-hydroxypentanoate (206)

To a suspension of (204) (110 mg, 0.61 mmol) in DMF (3 ml) was added ethyl iodide (0.20 ml, 3.65 mmol). The mixture was stirred at room temperature under a nitrogen atmosphere for 40 h. Excess ethyl iodide was evaporated *in vacuo*. The residue was diluted with water (3 ml) and extracted with ethyl acetate (3 x 5 ml). The organic layer was washed with water (5 ml), brine (5 ml) and then dried (Na_2SO_4). Evaporation of the solvent *in vacuo* left a brown oil which was purified by column chromatography (silica gel) to afford (R)-(-)-ethyl-5-azido-4-hydroxypentanoate (206) (68 mg, 60%) as a pale yellow oil. ν_{max} (thin film) 3400 (br, OH), 2110 (azido) and 1730 cm^{-1} (C=O, ester), δ_H (270 MHz; $CDCl_3$) 1.27 (3H, t, J = 7.14 Hz, $C\underline{H}_3CH_2O$), 1.72-1.85 (2H, m, $C\underline{H}_2CH_2CO$), 2.49 (2H, t, J = 7.14 Hz, $CH_2C\underline{H}_2CO_2C_5H_5$), 2.93 (1H, br, OH), 3.24-3.40 [2H, d of d, ABX system, J_{AB} = 12.41 Hz, $N_3C\underline{H}_2CH(OH)]$, 3.81 [1H, m, $N_3CH_2C\underline{H}(OH)]$ and 4.14 (2H, q, J = 7.15 Hz, $CH_3C\underline{H}_2O$), δ_C (270 MHz; $CDCl_3$) 173.97 (35% C1), 30.47 (100% C2), 29.14 (94% C3), 70.09 (96% C4), 56.92 (99% C5), 60.78 (68% $CH_3C\underline{H}_2O$) and 14.19 (78%, $C\underline{H}_3CH_2O$), m/z 188 (M+1), 170 ($M-H_2O$), 142 ($M-OC_2H_5$), 131 (100%) and 85 ($N_3C_2H_3O^+$).

Preparation of (S)-Ethyl-5-azido-4-hydroxypentanoate (205)

Like the R-isomer, this compound was prepared from (203). ν_{max} (thin film) 3400 (br, OH), 2110 (azido) and 1730 cm^{-1} (C=O, ester), δ_H (270 MHz; $CDCl_3$) 1.26 (3H, t, J = 7.15 Hz, $C\underline{H}_3CH_2O$), 1.75-1.85 (2H, m, $C\underline{H}_2CH_2CO$), 2.49 (2H, t, J = 7.14 Hz, $CH_2C\underline{H}_2CO_2Et$), 2.91 (1H, br, OH), 3.29-3.35 [2H, d of d, ABX system, J_{AB} = 12.27 Hz, $N_3C\underline{H}_2CH(OH)$], 3.81 [1H, m, $N_3CH_2\underline{C}H(OH)$], and 4.14 (2H, q, J = 7.14 Hz, $CH_3C\underline{H}_2O$). δ_C (270 MHz; $CDCl_3$) 173.84 (38% C1), 30.36 (70% C2), 29.02 (89% C3), 69.98 [78% $N_3CH_2\underline{C}H(OH)$], 56.83 (92% C5), 60.67 (64%, $CH_3\underline{C}H_2O$) and 14.08 (71%, $\underline{C}H_3CH_2O$), m/z 188 (M+1), 170 ($M-H_2O$), 142 ($M-OC_2H_5$), 131, 114 and 85.

Preparation of (S)-(-)-Methyl-5-azido-4-hydroxypentanoate (211)

(184) (4.84 g, 0.03 mol) was dissolved in methanol (94 ml). Sodium hydroxide (1.37 g, 0.03 mol) was added. The mixture was heated under reflux for 2 h, evaporated *in vacuo* and the solid residue was dissolved in water (10 ml) and extracted with ethyl acetate (2 x 10 ml). The aqueous layer was acidified to pH 4.5 with 2N hydrochloric acid and extracted with ethyl acetate (5 x 20 ml). The combined extracts were dried (Na_2SO_4) and evaporated to give a colourless viscous oil, a free acid, which was immediately treated with excess diazomethane in ether at 0 °C to give, after column chromatography (silica gel), (S)-(-)-methyl-5-azido-4-hydroxypentanoate (211) (3.17 g, 53%) as a pale yellow oil. $[\alpha]_D^{22}$ -10.43 (C, 1.83, $CHCl_3$), ν_{max} (thin film) 3480 (br, OH), 2110 (azido) and 1730 cm^{-1} (C=O, ester), δ_H (270 MHz; $CDCl_3$) 1.75-1.85 (2H, m, $C\underline{H}_2CH_2CO$), 2.50 (2H, t, J = 7.32 Hz, $CH_2C\underline{H}_2CO$), 3.24-3.27 (3H, ABX, J_{AB} = 12.46 Hz, $N_3C\underline{H}_2CHO\underline{H}$), 3.69 (3H, s, $CO_2C\underline{H}_3$) and 3.81 [1H, m, $N_3CH_2\underline{C}H(OH)$], δ_C (270 MHz; $CDCl_3$) 174.42 (C1), 30.18 (46% C2), 29.17

(56% C3), 69.98 (79% C4), 56.89 (94% C5) and 51.90 (26% OCH_3), m/z 174 (M+1), 156 (M-H_2O), 142 (M-CH_3OH), 117 and 85 (100%).

Preparation of (R)-(+)-Methyl-5-azido-4-hydroxypentanoate (212)

The preparation of this compound was analogous to that for the S-isomer. It was prepared from (188) as a pale yellow oil. $[\alpha]_D^{22}$ 10.16 (C 1.87, $CHCl_3$), ν_{max} (br, OH), 2110 (azido) and 1720 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 1.74-1.85 (2H, m, $C\underline{H}_2CH_2CO$), 2.49 (2H, t, J = 7.32 Hz, $CH_2C\underline{H}_2CO$), 3.24-3.37 [2H, d of d, AB, J_{AB} = 12.46 Hz, $N_3C\underline{H}_2CH(OH)$], 3.62 (1H, br, OH), 3.68 (3H, s, $CO_2C\underline{H}_3$) and 3.77-3.80 [1H, m, $N_3CH_2C\underline{H}(OH)$], δ_C (270 MHz; $CDCl_3$) 174.50 (31% C1), 30.16 (89% C2), 29.26 (92% C3), 69.95 (98% C4), 56.84 (100% C5) and 51.90 (43%, $CO_2C\underline{H}_3$), m/z 174 (M+1), 156 (M-H_2O), 142 (M-CH_3OH), 117 and 85.

Preparation of (R)-(+)-Methyl-(2-aziridinyl)-2-propanoate (223)

To a stirred solution of (S)-(-)-methyl-5-azido-4-hydroxypentanoate (211) (1.0 g, 5.78 mmol) in ether (25 ml), at room temperature, was added triphenylphosphine (1.51 g, 5.76 mmol) all in one portion. The mixture was heated under reflux for 50 min and was allowed to cool to room temperature overnight. The precipitated triphenylphosphine oxide was filtered and the filtrate was concentrated. Distillation of the residue at reduced pressure gave 0.4 g (51%) of colourless oil, corresponding to (R)-(+)-methyl-(2-aziridinyl)-2-propanoate (223). $[\alpha]_D^{25}$ 16.75 (C 4.18, $CHCl_3$), ν_{max} (thin film) 3300 (br, NH) and 1720 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 1.38 (1H, d of d, J = 2.94 Hz, HN-$C\underline{H}_aH_bCH$), 1.53-1.74 (2H, m, $C\underline{H}_2CH_2CO$), 1.76 (1H, d of d, J = 6.41 Hz, HN-$CH_a\underline{H}_bCH$), 1.79-2.03 (1H, m, $HN\text{-}CH_2C\underline{H}CH_2$), 2.46 (2H, t, J = 7.33 Hz, $CH_2C\underline{H}_2CO$) and 3.67 (3H, s, $CO_2C\underline{H}_3$), δ_C (270 MHz; $CDCl_3$) 173.66 (25% C1), 31.90 (61% C2), 29.42 (23% C3), 29.29 (16% CH) 24.93 (100%, $N\underline{C}H_2CH$) and 51.52 (65% $CO_2C\underline{H}_3$) m/z 130 (M+1), 116 and 69 (100%).

Preparation of(S)-(+)-Methyl-(2-aziridinyl)-2-propanoate (225)

Like the R-isomer, this compound was prepared in the same manner, from (R)-(-)-(212) in 58% yield as a colourless oil. ν_{max} (thin film) 3300 (br, NH) and 1725 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 1.38 (1H, d of d, J = 3.29 Hz, HN-C$\underline{H}_a$H$_b$CH), 1.58-1.77 (2H, m, C$\underline{H}_2$CH$_2$CO), 1.805 (1H, d, J = 6.41 Hz, HN-CH$_a$$\underline{H}_b$CH), 2.03-2.17 (1H, m, HN-CH$_2$C$\underline{H}$CH$_2$), 2.48 (2H, t, J = 7.32 Hz, CH$_2$$\underline{CH}_2$CO) and 3.68 (3H, s, $CO_2C\underline{H}_3$), δ_C (270 MHz; $CDCl_3$) 173.75 ($\underline{C}O_2CH_3$), 31.92 ($CH_2\underline{CH}_2CO_2$), 29.34 ($\underline{CH}_2CH_2CO$), 29.38 ($NCH_2\underline{CH}$), 25.06 ($N\underline{CH}_2CH$) and 51.65 ($CO_2\underline{CH}_3$), m/z 130 (M+1), $C_6H_{11}O_2N$ requires $\underline{M}$ 129.

Preparation of (R)-(+)-sodium-(2-aziridinyl)-2-propanoate (224)

To a stirred solution of (223) (0.16 g, 1.24 mmol) in methanol (2 ml) was added sodium hydroxide (0.05 g, 1.24 mmol) in water (0.5 ml). The mixture was heated under reflux for 25 min. The solvent was removed *in vacuo*. The residue was redissolved in water (3 ml) and extracted with chloroform (3 x 5 ml). The aqueous layer was evaporated *in vacuo* below 40 °C to give white crystals which corresponded to (R)-(-)-sodium-(2-aziridinyl)-2-propanoate (224) (0.16 g, 94%). $[\alpha]_D^{25}$ 5.19 (C 3.85, H_2O), ν_{max} (Nujol) 3360 (br, NH), 1550 cm^{-1} (CO_2Na), δ_H (270 MHz; D_2O) 0.625 (1H, d of d, J = 3.66 Hz, NHC$\underline{H}_a$H$_b$CH), 0.83-0.88 (2H, m, C$\underline{H}_2$CH$_2$CO), 1.04 (1H, d of d, J = 6.04 Hz, NH-CH$_a$$\underline{H}_b$CH), 1.25 (1H, m, NH-CH$_2C\underline{H}CH_2$) and 1.56 (2H, t, J = 7.60 MHz, CH$_2$$\underline{CH}_2$CO), δ_C (270 MHz; D_2O) 182.92 (C=O), 35.37 ($C\underline{H}_2CO_2Na$), 29.82 ($C\underline{H}_2CH_2CO_2Na$), 29.65 ($NHCH_2\underline{CH}$) and 24.26 ($N\underline{CH}_2CH$), m/z 138 (+) FAB $C_5H_8O_2NNa$ requires $\underline{M}$ 137. Due to the hygroscopic nature of this compound no satisfactory elemental analysis data could be obtained.

Preparation of (S)-(-)-Sodium-(2-aziridinyl)-2-propanoate (226)

The procedure for the preparation of this compound was similar to that for the R-isomer and was isolated in 62% yield. $[\alpha]_D^{25}$ -5.33 (C 3.75 H_2O), ν_{max} (Nujol) 3360 (br, NH) and 1560 cm^{-1} (CO_2Na), δ_H (270 MHz; D_2O) 0.625 (1H, d of d, J = 3.66 Hz, $NHCH_aH_bCH$), 0.83-0.89 (2H, m, $CH_2CH_2CO_2Na$), 1.04 (1H, d of d, J = 6.04 Hz, $NH\text{-}CH_aH_bCH$), 1.27 (1H, m, $NHCH_2CHCH_2$) and 1.56 (2H, t, J = 7.51 Hz, CH_2CH_2CO), δ_C (270 MHz; D_2O) 182.92 (C=O), 35.34 (CH_2CO_2Na), 29.81 (CH_2CH_2CO), 29.61 (NCH_2CH) and 24.23 ($NHCH_2CH$).

Due to the hygroscopic nature of this compound, no satisfactory elemental analysis data could be obtained.

Preparation of (S)-(-)-Methyl-5-azido-4-methanesulphonyloxy-pentanoate (216)

(S)-(-)-(211) (0.57 g, 3.29 mmol) was dissolved in pyridine and cooled to 0 °C. Methanesulphonyl chloride (0.40 g, 3.49 mmol) was added *via* a syringe and the mixture was stirred at this temperature under a nitrogen atmosphere overnight. The mixture was diluted with 2N hydrochloric acid (10 ml), extracted with ethyl acetate (3 x 20 ml) and washed the organic layer with water (50 ml) and then with brine (50 ml). After drying ($MgSO_4$), the solvent was removed at reduced pressure to give a yellow oil (0.87 g) which was purified by dry-column flash chromatography (silica gel) using ethyl acetate-petroleum ether as eluant to afford (S)-(-)-methyl-5-azido-4-methanesulphonyloxy-pentanoate (216) (0.74 g, 89%) as a pale yellow syrup, $[\alpha]_D^{23}$ -22.04 (C 4.99, $CHCl_3$), ν_{max} ($CHCl_3$) 2100 (azido) and 1730 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 2.04 (2H, q, J = 7.24 Hz, $CH_2CH_2CO_2$), 2.50 (2H, t, J = 7.33 Hz, CH_2CH_2CO), 3.12 (3H, s, SCH_3), 3.41-3.68 (2H, d of d, BAX, J_{AB} = 13.19 Hz, N_3CH_2CH), 3.69 (3H, s, CO_2CH_3) and 4.82-4.85 (1H, m, $N_3CH_2CHCH_2$), δ_C (270 MHz; $CDCl_3$) 172.81 (CO_2Me), 29.17 (C2)

27.36 (C3), 79.35 (C4), 54.00 (C5), 51.90 ($CO_2\underline{C}H_3$) and 38.50 (SCH_3), m/z 252 (M+1), 156 (M-CH_3SO_3H), $C_7H_{13}O_5N_3S$ requires M 251.

Preparation of (S)-5-Methanesulphonyloxy-2-piperidinone (220)

(216) (0.24 g, 0.96 mmol) and 10% palladium on carbon (100 mg) were stirred together in methanol (5 ml) at room temperature under a low pressure of hydrogen for 16 h. The catalyst was filtered and the filtrate was evaporated at reduced pressure. The residual colourless oil was twice purified by dry-column flashchromatography on silica gel using methanol-dichloromethane as eluant to afford (S)-5-methanesulphonyloxy-2-piperidinone (220) (70 mg, 27%) as a white crystalline product. ν_{max} (Nujol) 3200 (br, NH) and 1660 cm^{-1}(C=O, lactam), δ_H (270 MHz; $CDCl_3$) 2.12-2.53 (4H, m, $C\underline{H}_2C\underline{H}_2CO$), 3.15 (3H, s, $SC\underline{H}_3$), 3.55-3.64 (2H, m, NH-$C\underline{H}_2CH$), 5.10-5.13 (1H, m, $HNCH_2\underline{C}H$) and 3.30-3.31 (1H, NH), δ_C (270 MHz; $CDCl_3$) 173.41 (C2), 27.18 (23%, C3), 26.74 (28% C5), 74.36 (28% C4), 47.32 (25% C6) and 38.35 (33% $S\underline{C}H_3$). [Found: C, 38.0; H, 6.01; N, 7.23%; (M+1), 194 and 98 (M-CH_3SO_3H). $C_6H_{11}O_4NS$ requires C, 37.30; H, 5.74; N, 7.25%; M 193.2232].

Preparation of (S)-Methyl-5-dimethoxyphosphorylamino-4-hydroxypentanoate (229)

(211) (0.55 g, 3.18 mmol) was dissolved in benzene (20 ml) and trimethyl phosphite (2.6 ml, 21.94 mmol) was added *via* a syringe. The mixture was refluxed for 2 h under a nitrogen atmosphere. After removal of the solvent and excess reagent *in vacuo*, the residue was columned on silica gel eluting with methanol-dichloromethane to afford (S)-methyl-5-dimethoxyphosphorylamino-4-hydroxypentanoate (229) (0.6 g, 74%) as a colourless unstable oil. ν_{max} ($CHCl_3$) 3390 (OH), 3260 (NH) and 1740 cm^{-1} (C=O, ester), δ_H (270 MHz; $CDCl_3$) 2.07-2.31 (2H, m, $C\underline{H}_2CH_2CO_2CH_3$), 2.35-2.63 (2H, m, $\underline{C}H_2CO_2CH_3$), 3.41-3.89 [11H, m,

$P(O\underline{CH}_3)_2$, $CO_2\underline{CH}_3$, $NH\underline{CH}_2$] and 4.78-4.82 (1H, m, $NHCH_2\underline{CH}CH_2$), m/z 256 (M+1), 130 [M-$(CH_3O)_2P(O)NH_2$], 224 (M-MeOH) and 238 (M-H_2O), $C_8H_{18}O_6NP$ requires M 255.

Preparation of (S)-(-)-Methyl-4-methaneuslphonyloxy-5-trimethoxy-phosphoranylidenaminopentanoate (227)

(216) (0.34 g, 1.35 mmol) and trimethyl phosphite (0.19 g, 2.2 mmol) in ether (12 ml) were heated under reflux for 16 h under a stream of nitrogen. The solvent and excess reagent were evaporated *in vacuo*. The residual oil was purified by dry-column flash chromatography on silica gel using methanol-dichloromethane as eluant to afford (S)-(-)-methyl-4-methanesulphonyloxy-5-trimethoxyphosphoranylideneaminopentanoate (227) (0.24 g 51%) as a pale yellow oil. $[\alpha]_D^{23}$ -12.0 (C 5.0 $CHCl_3$), ν_{max} ($CHCl_3$) 3400 (OH) and 1740 cm^{-1} (C=C, ester), δ_H (270 MHz; $CDCl_3$) 1.63-1.76 (2H, m, $\underline{CH}_2CH_2CO_2CH_3$), 1.90-2.00 (2H, m, $CH_2\underline{CH}_2CO_2CH_3$), 3.23-3.43 (2H, m, $N\underline{CH}_2CH$), 3.77-3.81 [9H, d, J_{P-H} = 30.41 Hz, $P(OCH_3)_3$], 3.92 (3H, s, $CO_2\underline{CH}_3$), 3.03 (3H, s, $SC\underline{H}_3$) and 4.87 (1H, m, CH), δ_C (270 MHz; $CDCl_3$) 173.15 (C1), 27.37 (C3), 29.16 (C2), 38.50 (SCH_3), 51.70 [3 x $P(OCH_3)_3$], 51.81 ($CO_2\underline{CH}_3$), 54.00 (N-$\underline{CH}_2CH$) and 79.63 (C4), m/z 348 (M+1), 224 (M-C_3H_9OP), 252 (M-CH_3SO_3H), $C_{10}H_{22}O_8NPS$ requires M 347.

Preparation of Ethylbut-3-enoate (233)

But-3-enoic acid (5.0 g, 58.14 mmol) was dissolved in dry ether (50 ml) and *N,N*-dimethylformamide (0.5 ml) was added. The mixture was cooled to 0 °C and stirred under a nitrogen atmosphere. Thionyl chloride (6.4 ml, 1.5 equivalents) was added over 5 min and stirring was continued for 3 h. An insoluble brown oil precipitated out. The solvent and excess thionyl chloride were evaporated off *in vacuo* at room temperature. The residual oil was dissolved in dry ethanol (10 ml) and cooled to 0 °C under an atmosphere of nitrogen. Sodium

(1.49 g, 64.78 mmol) in ethanol (30 ml) was added with stirring over 15 min. After addition, the mixture became a white suspension and was allowed to warm to room temperature overnight. Water (50 ml) and dichloromethane (100 ml) were added. After separating the two layers, the aqueous layer was extracted with dichloromethane (2 x 100 ml) and the combined organic layer was washed with saturated sodium bicarbonate (2 x 50 ml) and then with water (100 ml), dried (Na_2SO_4) and evaporated *in vacuo* to give 3.59 g of dark brown oil which was quite pure by T.L.C. Short-path distillation at reduced pressure afforded 2.35 g (35%) of pale yellow oil. ν_{max} (thin film) 1730 (C=O, ester), 1180 (C-O) and 1670 cm^{-1} (C=C), m/z 69 ($M-C_2H_5O$), 115 (M+1).

Preparation Ethyl-4-amino-3-bromobutanoate (235)

To a stirred ethylbut-3-enoate (233) (0.5 g, 4.38 mmol) at 0 °C was added bromine (0.70 g, 4.38 mmol) in dichloromethane (3 ml) over 3 min. After 10 min, the mixture was allowed to warm to room temperature over 2 h. The solvent was removed at reduced pressure, room temperature and the residue was dissolved in THF(2 ml). Aqueous ammonia (0.15 g, 4.38 mmol) in THF (5 ml) was cautiously added and a white precipitate immediately formed. After stirring for 30 min, the solvent was evaporated and the residue was taken up in chloroform (10 ml) and filtered through a pad of celite. The filtrate was evaporated and the oil obtained was subjected to column chromatography on silica gel using ethanol-dichloromethane (1:7 v/v) as eluant to afford ethyl-4-amino-3-bromobutanoate (235) (0.44 g, 48%) as a colourless oil. ν_{max} (thin film) 3460 (br, NH) and 1735 cm^{-1} (C=O, ester), m/z 195, 193 ($M-NH_3$), 167, 165 ($M-OC_2H_5$) and 85 (100%).

Reaction of Ethyl-4-amino-3-bromobutanoate (235) with sodium hydroxide

(235) (0.25 g, 1.19 mmol) in methanol (0.8 ml) was mixed with sodium hydroxide (0.23 g, 5.75 mmol) in water (1.5 ml). After stirring the mixture for 1 h, the solvent was removed at reduced pressure to leave white crystals.* Analysis of these crystals with NMR and IR spectroscopy revealed no presence of the desired product. ν_{max} (Nujol) 3580 cm^{-1} (br, NH), 1560 cm^{-1} ($CO_2^{-+}Na$) and 1640 cm^{-1} (C=C).

Preparation of Ethyl-4-azido-3-iodobutanoate (236)

Iodine chloride (0.72 g, 4.44 mmol) in dry acetonitrile (3 ml) was added to a stirred slurry of sodium azide (0.59 g, 9.08 mmol), also in acetonitrile (20 ml) at 0 °C under an atmosphere of nitrogen. After 10 min ethylbut-3-enoate (233) (0.5 g, 4.38 mmol in acetonitrile (4 ml) was added *via* a syringe. The mixture was allowed to warm to room temperature and stirred for 14 h. Water (30 ml) was added and the mixture was extracted with dichloromethane (3 x 30 ml). The combined organic layer was washed with 5% sodium thiosulphate (20 ml) and then water (30 ml), dried ($MgSO_4$) and evaporated *in vacuo* to give 0.25 g of dark brown oil which was found to consist of several spots on TLC. Column chromatography (neutral alumina) gave 6.5 mg of colourless oil, which quickly darkened on standing and broke into several spots on TLC. ν_{max} (thin film) 1730 (C=O) and 2100 cm^{-1} (azido). Because of the instability, no accurate mass could be obtained for this compound.

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*Acidification of the aqueous solution of these crystals with 2 N hydrochloric acid gave an oil which was found to contain at least 10 components by T.L.C. analysis.

Attempted Preparation of Methyl-3,4-epoxybutanoate (238) and Preparation of Methyl-4-hydroxybut-2-enoate (239)

4-Iodomethyloxetan-2-one (195) (1.14 g, 5.38 mmol) was dissolved in dry methanol (10 ml). Potassium carbonate (2.23 g, 16.13 mmol) was added all in one portion and the mixture was stirred at room temperature under a nitrogen atmosphere for 3 h (TLC). The solvent was evaporated at reduced presusre and the residue was diluted with a saturated brine solution (20 ml). Extraction of this mixture with ether and dichloromethane only gave a small amount of oil (200 ml), which was found to consist of several spots on TLC, with one major spot. Column chromatography of the mixture on silica gel using ethyl acetate-petroleum ether as eluant yielded 120 mg of pale yellow oil which corresponded to methyl-4-hydroxybut-2-enoate (239), a ring-opened product of the epoxide (238). ν_{max} ($CHCl_3$) 3400 (OH), 1730 (C=O) and 1650 cm^{-I} (C=C), δ_H (270 MHz; $CDCl_3$) 4.32 (2H, d, J = 1.83 Hz, $HO\underline{CH_2}CH$), 3.74 (3H, s, $CO_2\underline{CH_3}$), 3.74-3.75 (1H, m, $\underline{O}H$), 6.09 (1H, d of m, J_{AB} = 17.96 Hz, J_{trans} = 2.2 Hz, J_{cis} = 1.46 Hz, J = 0.74 Hz, $HC=\underline{C}HCO_2CH_3$) and 7.07 (1H, d of m, J_{BA} = 15.75 Hz, $\underline{H}C=CHCO_2CH_3$), δ_C (270 MHz; $CDCl_3$) 167.38 (28%, $\underline{C}O_2CH_3$), 119.34 (81%, $C\underline{H}CO_2CH_3$), 147.98 (98% $HOCH_2C\underline{H}$), 61.37 (63%, $HO\underline{CH_2}CH$) and 51.73 (100%, $CO_2\underline{CH_3}$), m/z 85 ($M-OCH_3$), 98 ($M-H_2O$) and 117 (M+1).

Preparation of Benzylbut-3-enoate (240)

But-3-enoic acid (5 g, 58.14 mmol) was dissolved in dry benzene (150 ml). Benzyl alcohol (6.28 g, 58.15 mmol) and *p*-toluenesulphonic acid monohydrate (2 g, 10.5 mmol) were added each in one portion. The mixture was heated under reflux in a Dean and Stark apparatus for 5 days, cooled to room temperature and stirred overnight. Water (100 ml) was added and the two layers were separated. The aqueous layer was

washed once with ether (100 ml). The combined organic layer was washed once with 10% sodium carbonate (50 ml), water (50 ml), brine (50 ml) and dried ($MgSO_4$). Filtration through a pad of silica gel afforded a brown oil (10.91 g) which was quite pure by TLC analysis. ν_{max} ($CHCl_3$) 1730 (C=O, ester) and 1600 cm^{-1} (aromatic), m/z 91 (100% M-$C_6H_5CH_2$) and 176 (M).

Benzyl-3,4-epoxybutanoate (241)

To (240) (3.3 g, 18.75 mmol) in dichloromethane (30 ml) under a nitrogen atmosphere at room temperature was added, *via* a pressure-equalising dropping funnel, a suspension of *m*-chloroperbenzoic acid (3.33 g, 19.25 mmol) in dichloromethane (50 ml) over 20 min. The resulting clear solution was stirred at room temperature for 3 days. 10% sodium sulphite (100 ml) was added and the two layers were separated. The organic layer was washed with 5% sodium bicarbonate (2 x 20 ml), then with water (50 ml) and finally with brine (2 x 50 ml), dried ($MgSO_4$) and evaporated *in vacuo* to give 3.26 g of yellow oil. Dry-column flash chromatography on silica gel using ethyl acetate-petroleum ether as eluant afforded 2.87 g (80%) of colourless oil. ν_{max} ($CHCl_3$) 1730 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 2.44-2.47 (1H, m, OCH_aH_bCH), 2.52-2.54 (2H, m, $CHCH_2CO_2$), 2.71-2.74 (1H, m, OCH_aH_bCH), 3.21-3.22 (1H, m, $OCHCH_2$), 5.11 (2H, s, $CH_2C_6H_5$) and 7.30 (5H, s, $C_6H_5CH_2$), m/z 91 (M-$PhCH_2$), 163, 187 and 193 (M+1).

Preparation of Benzyl-3-chloro-4-ethoxyformamidobutanoate (249)

(240) (5.70, 32.28 mmol) in dry benzene (30 ml) was added to a stirred solution of *N,N*-dichlorourethane (5.10 g, 32.28 mmol) in benzene (30 ml) under a nitrogen atmosphere at room temperature. The mixture was then heated under reflux for 20 h, cooled to 0 °C and was diluted with 20% sodium thiosulphate (100 ml). After stirring for 30 min, the

two layers were separated and the aqueous layer was washed once with ether (100 ml). The combined organic layer was washed with water (100 ml) and then brine (2 x 50 ml), dried ($MgSO_4$) and evaporated *in vacuo* to give a residue which was purified by column chromatography (silica gel) to afford benzyl-3-chloro-4-ethoxyformamidobutanoate (249) (5.37 g, 62%) as a pale yellow oil. ν_{max} ($CHCl_3$) 3440 (NH) and 1720 (C=O, br), δ_H (270 MHz; $CDCl_3$) 1.22 (3H, t, J = 7.14 Hz, $C\underline{H}_3CH_2O$), 2.75 (1H, d of d, J_{AB} = 16.31 Hz and J_{AX} = 8.62 Hz, α-proton), 2.87 (1H, d of d, J_{AB} = 16.31 Hz and J_{BX} = 4.95 Hz, α-proton), 3.49 (2H, d of d, J = 6.22 Hz, $NH\underline{CH}_2CHCl$), 4.09 (2H, q, J = 7.32 Hz, $CH_3\underline{CH}_2O$), 4.40 (1H, m, β-proton), 5.14 (2H, s, $\underline{CH}_2C_6H_5$), 5.50 (1H, br, NH) and 7.33 (5H, s, $CH_2C_6\underline{H}_5$), δ_C (270 MHz; $CDCl_3$) 14.55 (36%, $\underline{CH}_3CH_2O$), 40.50 (20% $\underline{CH}_2CO_2$-CH_2Ph), 46.63 (20%, NH-$\underline{CH}_2CHCl$), 56.65 (20% $CH_2\underline{CH}Cl$), 61.14 (16% $CH_3\underline{CH}_2O$), 66.83 (55% $\underline{CH}_2C_6H_5$), 156.73 (8% $NH\underline{CO}_2C_2H_5$), 169.64 (27% $\underline{CO}_2CH_2C_6H_5$), 135.46, 128.25, 128.28, 128.33, 128.36 and 128.57 (aromatic carbons). [Found: $\underline{M}^+$ 299.0852, $C_{14}H_{18}O_4NCl$ requires $\underline{M}$, 299.0877].

Preparation of Benzyl-4-ethoxyformamidobut-2-enoate (255)

To a stirred solution of (249) 0.86 g, 2.88 mmol) in dichloromethane (30 ml) at 0 °C under a nitrogen atmosphere was added 1,8-diazabicyclo-[5.4.0]undec-7-ene (0.44 g, 2.88 mmol) *via* a syringe. After 3½ h, the mixture was evaporated *in vacuo* and subjected to column chromatography (silica gel) to afford 0.35 g (46%) of colourless viscous oil from a complex mixture of products. This was found to be benzyl-4-ethoxyformamidobut-2-enoate (255). ν_{max} ($CHCl_3$) 3460 (NH), 1710 (C=O, br) and 1660 cm^{-1} (C=C), δ_H (270 MHz; $CDCl_3$) 1.22 (3H, t, J = 7.14 Hz, $C\underline{H}_3CH_2O$), 3.93 (2H, br, $NH\underline{CH}_2CH$), 4.11 (2H, q, J = 7.15 Hz, $CH_3\underline{CH}_2O$), 5.16 (2H, s, $\underline{CH}_2C_6H_5$), 5.98 (1H, d of t, J_{AB} = 15.76 Hz, α-proton), 6.94 (1H, d of t, J_{AB} = 15.75 Hz and J = 4.76 Hz, β-proton) and 7.34 (5H, s, $CH_2C_6\underline{H}_5$), δ_C (270 MHz; $CDCl_3$) 165.87 (12%, $\underline{CO}_2CH_2Ph$), 156.50

(10%, $NCO_2C_2H_5$), 145.28 (28%, C_β), 120.98 (14%, C_α), 66.33 (44%, $\underline{C}H_2C_6H_5$), 61.14 (10%, $CH_3\underline{C}H_2O$), 14.56 (20%, $\underline{C}H_3CH_2O$), 128.28, 128.57 and 135.84 (aromatic carbons), m/z 263 (M^+), 192, 157, 129, 107 and 91 (100%).

Preparation of Sodium 4-ethoxyformamidobutanoate (257)

(255) (1.06 g, 4.03 mmol), 10% palladium on carbon (0.3 g) and sodium bicarbonate (0.3 g, 3.57 mmol) were stirred in THF-water (15:10 ml) at room temperature under a pressure of hydrogen for 50 h. The catalyst was filtered through a pad of celite and was washed with methanol. The filtrate was concentrated *in vacuo* and diluted with water (10 ml). The mixture was extracted with chloroform (3 x 25 ml) and the aqueous layer was evaporated to dryness at reduced pressure to give 0.69 g of white crystals which corresponded to sodium 4-ethoxyf o rmamidobutanoate (257). δ_H (270 MHz; D_2O) 0.99 (3H, t, J = 6.5 Hz, $C\underline{H}_3CH_2O$), 1.48 (2H, m, $\underline{C}H_2CH_2CO_2Na$), 1.98 (2H, t, J = 7.88 Hz, $\underline{C}H_2CO_2Na$), 2.88 (2H, t, J = 6.96 Hz, $HN\underline{C}H_2$) and 3.85 (2H, q, J = 7.15 Hz, $CH_3\underline{C}H_2O$), δ_C (270 MHz; D_2O) 14.01 ($\underline{C}H_2CH_2O$), 26.05 ($HN\underline{C}H_2CH_2$), 32.95 ($\underline{C}H_2CH_2CO_2Na$), 34.77 ($\underline{C}H_2CO_2Na$), 61.53 ($CH_3\underline{C}H_2O$), 158.64 ($HN\underline{C}O_2C_2H_5$) and 182.68 ($\underline{C}O_2Na$), m/z 194 (-) FAB, 172 (M-Na).

Reaction of Benzyl-3-chloro-4-ethoxyformamidobutanoate (249) with sodium hydride*

To a stirred suspension of sodium hydride (0.152 g, 0.63 mmol) in *N,N*-dimethylformamide (5 ml) under a nitrogen atmosphere at room temperature was added (249) (0.19 g, 0.63 mmol). The mixture was stirred at 40 °C for 20 h, quenched with a saturated solution of ammonium chloride (10 ml) and was extracted with ether (3 x 10 ml). The organic layer was washed

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*When two equivalents of sodium hydride were used a complex mixture of products resulted. The crude product still contained benzyl alcohol.

with water (10 ml) and then with brine (10 ml), dried ($MgSO_4$) and evaporated to give 80 mg of yellow oil. This was found to be a complex mixture of products from which 20 mg of colourless oil was isolated together with benzyl alcohol by column chromatography (silica gel). This oil was found to be the dehydrochlorinated product of the starting compound and corresponded to (255). ν_{max} (thin film) 3440 (NH), 1720 (C=O, br) and 1650 cm^{-1} (C=C), m/z 264 (M+1), 91 (100%), 173 ($M-C_6H_7$) and 218 (M-EtOH).

Reaction of Benzyl-3-chloro-4-ethoxyformamidobutanoate (249) with Potassium hydroxide*

(249) (5.02 g, 17.0 mmol) in ethanol (130 ml) was mixed with potassium hydroxide (8.46 g, 150 mmol) in water (35 ml). The mixture was heated at 60 °C for 21 h. The analysis on silica gel still indicated the presence of the starting material. The temperature was raised by heating the mixture under reflux for 1 day. This time all the starting material was consumed (TLC). Ethanol was evaporated *in vacuo* and the aqueous residue was extracted with chloroform (3 x 30 ml), dried ($MgSO_4$) and evaporated to leave 1.3 g of yellow oil, which was a mixture of benzyl alcohol and several other components, but contained no desired product. The aqueous layer was freeze-dried to leave white crystals. Proton NMR spectrum of these crystals in D_2O did not indicate the presence of any ring-protons of the aziridine, but only a mixture of other unidentified products. Since fractional crystallisation was not possible, the crystals were redissolved in water (20 ml) and acidified to pH 6.67 with 2N hydrochloric acid. Extraction of this mixture with chloroform (3 x 20 ml) after saturation with brine gave a small amount of oil (100 mg) which was found to be a complex mixture

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*When one equivalent of potassium hydroxide was used only the benzyl ester was hydrolysed.

of products on TLC.

The aqueous layer was further reacidified to pH 5.0 and was evaporated *in vacuo* to dryness. The residual solid was trituated with chloroform to get 150 mg of yellow oil, which was found to be a complex mixture of products.

Preparation of Benzyl-4-azido-3-iodobutanoate (243)

To a stirred suspension of sodium azide (1.82 g, 0.03 mol) in acetonitrile (20 ml) at 0 °C under a nitrogen atmosphere was added a solution of iodine chloride (2.23 g, 0.01 mol) in acetonitrile (20 ml) over 10 min. After stirring for 15 min, benzylbut-3-enoate (240) (1.00 g, 5.68 mmol) in acetonitrile (15 ml) was added. After 15 min the mixture was allowed to warm to room temperature. After 3 days the mixture was poured into water (150 ml) and was extracted with ether (3 x 100 ml), washed with 5% sodium thiosulphate (1 x 130 ml), with water (2 x 100 ml) and finally with brine (100 ml). After drying ($MgSO_4$) and filtration through alumina, the solvent was evaporated *in vacuo* to afford 1.56 g (79%) of brown unstable oil which was quite pure by TLC (alumina). ν_{max} ($CHCl_3$) 2100 (azido) and 1730 cm^{-1} (C=O, ester), δ_H (270 MHz; $CDCl_3$) 2.53 (1H, d of d, J_{AB} = 16.58 Hz, $CHIC\underline{H}_aH_bCO$), 2.71 (1H, d of d, J_{AB} = 16.58 Hz, $CHICH_a\underline{H}_bCO$), 2.88-3.12 (1H, m, X, $CH_2C\underline{H}ICH_2$), 3.18 (1H, d of d, J_{AB} = 8.56 Hz, $N_3C\underline{H}_xH_yCHI$), 3.24 (1H, d of d, J_{AB} = 8.56 Hz, $N_3CH_x\underline{H}_yCHI$), 5.12 (2H, s, $C\underline{H}_2C_6H_5$) and 7.32 (5H, $CH_2C_6\underline{H}_5$), δ_C (270 MHz; $CDCl_3$) 169.54 (30%, $\underline{C}O_2CH_2C_6H_5$), 39.41 (60%, C2), 58.71 (25%, C3), 50.99 (5%, C4), 66.82 (55%, $C\underline{H}_2C_6H_5$), 135.29, 128.83, 128.54, 128.28, 128.15 and 128.09 (aromatic carbons). Because of the instability of this compound, no molecular mass measurement was possible. However, there were prominant peaks in the mass spectrum at 91 (base), 108, 131, 159, 168, 196, 197 and 232. There was no molecular ion.

Reaction of Benzyl-4-azido-3-iodobutanoate (243) with lithium aluminium hydride

(243) (0.41 g, 1.19 mmol) in ether (8 ml) was added to a suspension of lithium aluminium hydride (0.10 g, 2.63 mmol) in ether(30 ml) at 0 °C under a nitrogen atmosphere. The mixture was stirred at this temperature for 30 min and was then allowed to reach room temperature. After 15 h, 20% sodium hydroxide (2 ml) was added and the white precipitate formed was filtered through celite and dried ($MgSO_4$) to give 0.32 g of yellow oil. This was found to be a mixture of several products by TLC analysis. Benzyl alcohol and a small amount of starting compound were isolated from the mixture by column chromatography (neutral alumina). No desired product could be found from other fractions.

Reaction of Benzyl-4-azido-3-iodobutanoate (243) with Triphenylphosphine

(243) (0.73 g, 2.12 mmol) was dissolved in ether (50 ml) and was kept stirring under a pressure of nitrogen. Triphenylphosphine (0.55 g, 2.11 mmol) was added. The mixture was stirred for 8½ h at room temperature. The solvent was evaporated *in vacuo*. Analysis of the residue by IR spectroscopy indicated the absence of an absorption band at 2100 (azido) and the presence of a band at 1650 cm^{-1} (C=C). TLC analysis on both alumina and silica gel revealed a large number of products. Proton NMR spectrum of this mixture revealed no desired product. No attempt was made to separate this mixture.

Attempted Preparation of Benzyl-4-azido-3-hydroxybutanoate (237b) and Preparation of Benzyl-4-hydroxybut-2-enoate (242)

The epoxide (241) (1.32 g, 6.87 mmol) was dissolved in *N,N*-dimethylformamide (50 ml) and sodium azide (2.68 g, 41.25 mmol) was added all at once. The mixture was stirred at 60 °C for 3 h under a nitrogen

atmosphere, diluted with water (100 ml) and extracted with ethyl acetate (3 x 100 ml). The organic layer was washed with saturated brine (100 ml), dried ($MgSO_4$) and evaporated at reduced pressure to leave 1.14 g of yellow oil which contained a lost of *N,N*-dimethylformamide. TLC analysis indicated a large number of products. Column chromatography gave 50 mg of colourless oil which corresponded to benzyl-4-azido-3-hydroxybutanoate (237b). ν_{max} ($CHCl_3$) 2100 (azido) and 1730 cm^{-1} (C=O, ester), m/z 91 (100%), 236 (M+1) and 0.5 g of pale yellow oil which corresponded to benzyl-4-hydroxybut-2-enoate (242). ν_{max} ($CHCl_3$) 3400 (OH), 1700 (C=O) and 1650 cm^{-1} (C=C), δ_H (270 MHz; $CDCl_3$) 3.21 (1H, br, $\underline{H}OCH_2$), 4.25 (2H, d of d, $HOC\underline{H}_2CH$), 5.15 (2H, s, $\underline{C}H_2C_6H_5$), 6.12 (1H, d of t, J_{AB} = 15.76 Hz and J = 2.2 Hz, H_α), 7.03 (1H, d of t, J_{BA} = 15.57 Hz and J = 3.89 Hz, H_β) and 7.33 (5H, $CH_2C_6\underline{H}_5$), δ_C (270 MHz; $CDCl_3$) 166.52 (26%, $\underline{C}O_2CH_2C_6H_5$), 148.15 (40%, $\underline{C}HCHCO_2R$), 119.49 (38%, $CH\underline{C}HCO_2R$), 61.55 (52%, $HOC\underline{H}_2CH$), 66.31 (75% $\underline{C}H_2C_6H_5$), 128.15, 128.23, 128.51, 128.54, 128.59 and 135.82 (aromatic carbons), m/z 91 (100%) 108, 162 and 192 (M+1).

Reaction of Benzyl-4-azido-3-hydroxybutanoate (237b) with Triphenylphosphine

The substrate (237b) (0.66 g, 2.81 mmol) was dissolved in ether (10 ml) at room temperature. To this was added triphenylphosphine (0.73 g, 2.81 mmol). Evolution of a gas was observed. The mixture was heated under reflux for 30 min. after which time the reaction was complete (IR). TLC analysis on silica gel and alumina showed a complex mixture of products. No further separation of this mixture was undertaken.

Preparation of But-3-enylacetate (258)

To a stirred solution of 3-buten-1-ol (1.0 g, 13.89 mmol) in dichloromethane (30 ml) at room temperature, were added acetic anhydride (2.13 g, 20.88 mmol) and pyridine (3.29 g, 41.64 mmol). The mixture was stirred for 18 h. Water (50 ml) was added and the two layers were separated. The aqueous layer was extracted with dichloromethane (50 ml) and the combined organic layer was washed with water (50 ml), saturated copper sulphate (60 ml) and finally with saturated brine (70 ml), dried ($MgSO_4$) and filtered through a pad of silica gel. Careful removal of the solvent *in vacuo* afforded pure (TLC) but-3-enylacetate (258) (1.38 g, 87%) as a colourless oil. ν_{max} (thin film) 1740 (C=O) and 1640 cm^{-1} (C=C), δ_H (270 MHz; $CDCl_3$) 2.01 (3H, s, $\underline{CH_3}CO$), 2.36 (2H, m, $CHC\underline{H_2}CH_2O$), 4.09 (2H, t, J = 7.78 Hz, $CH_2C\underline{H_2}O$), 5.08 (2H, m, $\underline{H_2}C=CHCH_2$) and 5.78 (1H, m, $H_2C=\underline{CH}CH_2$), δ_C (270 MHz; $CDCl_3$) 170.71 (25%, C=O), 134.28 (66%, $H_2C=\underline{C}HCH_2$), 117.19 (51%, $\underline{H_2}C=CHCH_2$), 63.48 (57%, $CH_2\underline{CH_2}O$), 33.28 (100%, $CH\underline{CH_2}CH_2O$) and 20.82 (40%, $\underline{CH_3}CO$), m/z 115 (M+1), 95, 73 and 62.

Preparation of 3-Chloro-4-ethoxyformamidobutyl acetate (262)

(258) (2.04 g, 17.89 mmol) was dissolved in dry benzene (50 ml) and was stirred under an atmosphere of nitrogen. *N,N*-dichlorourethane (3.30 g, 20.92 mmol) was added *via* a syringe and the mixture was heated under reflux for 20 h, cooled to 0 °C and diluted with 20% sodium thiosulphate (50 ml). After stirring for 30 min, the two layers were separated. The aqueous layer was extracted with ether (50 ml). The combined organic layer was washed with water (50 ml), brine (30 ml) and dried ($MgSO_4$). Column chromarography (silica gel) afforded 3.66 g (86%) of colourless oil corresponding to 3-chloro-4-ethoxyformamidobutyl acetate (262). ν_{max} (thin film) 3340 (NH), 1720-1740 (C=O) and 760 cm^{-1} (C-Cl), δ_H (270 MHz;

$CDCl_3$), 1.25 (3H, t, J = 7.15 Hz, $C\underline{H}_3CH_2O$), 1.82-2.23 (5H, m, $C\underline{H}_3CO$ and $CHCl\underline{CH}_2CH_2O$, AB system), 3.34-3.63 (2H, m, AB system, $HNC\underline{H}_2CHCl$), 4.06-4.30 (5H, m, $CH_2C\underline{H}Cl$, $CH_3C\underline{H}_2O$ and $CHClCH_2C\underline{H}_2O$) and 5.76 (1H, br, NH*), δ_C (270 MHz; $CDCl_3$) 173.93 (19%, $CH_3\underline{C}O$), 156.86 (24%, $\underline{C}O_2C_2H_5$), 61.04 (67%, $CH_3C\underline{H}_2O$), 61.11 (86%, $CHClCH_2\underline{C}H_2O$), 58.97 (64%, CHCl), 47.35 (69%, $NHC\underline{H}_2CHCl$), 34.28 (78%, $CHCl\underline{CH}_2CH_2O$), 20.86 ($\underline{C}H_3CO$) and 14.60 (73%, $\underline{C}H_3CH_2O$), m/z 238 (M+1), 202 (M-HCl), 192 ($M-C_2H_5O$), 178 ($M-CH_3CO_2H$), 141 and 102.

Preparation of 4-Azido-3-iodobutyl acetate (259) and 4-Hydroxy-3-iodobutyl acetate (260)

Sodium azide (9.57 g, 0.15 mol) was suspended in dry acetonitrile (100 ml) and cooled to 0 °C under an atmosphere of nitrogen. Iodine chloride (11.73 g, 0.07 mol) in acetonitrile (50 ml) was added. After stirring the mixture for 45 min, but-3-enylacetate (258) (1.0 g, 8.77 mmol) in acetonitrile (50 ml) was added. Stirring was continued for 2 h and the reaction was allowed to warm to room temperature. After 3 days, water (100 ml) was added and the whole was extracted with dichloromethane (3 x 150 ml). To aid extraction, 5% sodium thiosulphate solution (150 ml) was added and a vigorous reaction with evolution of a gas was observed until the solution became clear. The organic layer was washed with water (100 ml) and dried ($MgSO_4$). Filtration through neutral alumina gave 3.74 g of colourless oil which quickly darkened on standing. This was purified by column chromatography (silica gel) to afford 4-azido-3-iodobutyl acetate (3.34 g, 51%) as a colourless oil, but darkened on standing in the light. ν_{max} (thin film) 2100 (azido) and 1730 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 1.77-2.06 (5H, m, $C\underline{H}_3CO$ and $CHI\underline{CH}_2CH_2O$),

*The NH proton did not exchange with D_2O on deuteration.

3.34-3.36 (2H, m, AB, $NC\underline{H}_2CHI$), 3.64-3.80 (1H, m, $CH_2C\underline{H}ICH_2$) and 4.14-4.20 (2H, m, $CH_2C\underline{H}_2OCO$), δ_C (270 MHz; $CDCl_3$) 170.41 (22%, $CH_3\underline{C}O$), 60.56 (96%, $CH_2\underline{C}H_2O$), 59.55 (95%, $CH_2\underline{C}HI$), 35.81 (25%, $N_3\underline{C}H_2CHI$), 33.54 (63%, $C\underline{H}_2CH_2O$) and 20.82 (60%, $C\underline{H}_3CO$), m/z 284 (M+1), 241 (M-CH_3O), 244, 196, 181, 156 (M-HI), 87 and 85 (100%).

Another minor fraction gave 0.47 g of yellow oil which also darkened on standing and corresponded to 4-hydroxy-3-iodobutyl acetate (260). ν_{max} (thin film) 3420 (OH, br) and 1720 cm^{-1} (C=O), δ_H (270 MHz; $CDCl_3$) 3.23-3.39 (2H, m, $HOC\underline{H}_2CHI$, AB, J_{AB} = 10.26 Hz), 3.09 (1H, br, OH, disappeared on the addition of D_2O), 2.07 (3H, s, $\underline{C}H_3CO$), 1.74-2.03 (2H, m, $CHIC\underline{H}_2CH_2O$), 3.64-3.72 (1H, m, $HOCH_2C\underline{H}I$) and 4.10-4.25 (2H, m, $CH_2\underline{C}H_2O$), m/z 259 (M+1), 241 (M-H_2O) and 131 (M-HI).

Preparation of (2-Aziridinyl)ethanol (246)

Method 1. To a stirred solution of 3-chloro-4-ethoxyformamidobutyl acetate (262) (1.54 g, 6.50 mmol) in ethanol (800 ml) was added potassium hydroxide (3.27 g, 58.39 mmol) in water (20 ml). The mixture was heated at 50-60 °C for 2 days. Ethanol was evaporated off at reduced pressure and the aqueous residue was freeze-dried to leave a crystalline residue which was triturated with chloroform and dried ($MgSO_4$) to give 0.51 g of colourless smelly oil. This was found to be a complex mixture of products by T.L.C. analysis. Column chromatography (neutral alumina) gave 0.13 g (23%) of colourless oil as the main fraction. This corresponded to (2-aziridinyl)ethanol (246), δ_H (270 MHz; $CDCl_3$) 1.46 (1H, d of d, J = 3.66 Hz, $HN\text{-}\underline{C}H_aH_bCH$), 1.50-1.53 (1H, m, $CHC\underline{H}HCH_2$), 1.83 (1H, d of d, J = 6.05 Hz, $HNCH_a\underline{H}_bCH$), 1.86-1.94 (1H, m, $CHCH\underline{H}CH_2O$), 2.15 (1H, m, $HNCH_2C\underline{H}CH_2$) and 3.72 (2H, d of t, J = 5.96 Hz, $CH_2\underline{C}H_2O$), δ_C (270 MHz; $CDCl_3$) 23.99 (90%, $N\underline{H}_2CH$), 28.01 (63%, CH), 35.37 (38%, $\underline{C}H_2CH_2OH$) and 60.44 (100%, $CH_2\underline{C}H_2OH$), m/z 86 (M-1), 56 (base, M-$CH_2{=}\overset{+}{O}H$) and 75 (M-C^+).

The NH and OH protons were not observed.

Method 2. Lithium aluminium hydride (1.09 g, 28.67 mmol) was suspended in dry THF (100 ml) under nitrogen at room temperature. 4-Azido-3-iodobutyl acetate (259) (2.09 g, 7.38 mmol) in THF (20 ml) was added dropwise over 5 min. The mixture was stirred for 40 h, cooled to 0 °C and 20% sodium hydroxide solution (6 ml) was added. The resultant white precipitate was filtered through celite. The filtrate was dried ($MgSO_4$) and evaporated to leave 0.6 g of colourless gum which was found to consist of several spots on T.L.C. (alumina or silica gel). Column chromatography (alumina) gave only 50 mg (8%) of colourless oil which corresponded to (2-aziridinyl)ethanol (246). δ_H (270 MHz; $CDCl_3$) 1.47 (1H, d of d, J = 3.66 Hz, HN-C$\underline{H}_a$H$_b$CH), 1.70 (2H, m, CHC$\underline{H}_2$CH$_2$OH), 1.84 (1H, d of d, J = 5.87 Hz, NCH$_a$H$_b$CH), 2.12-2.19 (1H, m, NCH$_2$C$\underline{H}$), 3.52 (1H, br, NH), 3.62 (1H, t, J = 5.87 Hz, OH) and 3.78 (2H, m, CH$_2$C$\underline{H}_2$OH), δ_C (270 MHz; $CDCl_3$) 23.71 (28%, NHC$\underline{H}_2$CH), 28.02 (77%, NCH$_2$C$\underline{H}$), 35.32 (63%, C$\underline{H}_2$CH$_2$OH) and 60.49 (73%, CH$_2$C$\underline{H}_2$OH) m/z 86 (M-1), 56 (base, M-CH $=\overset{+}{O}$H), 71, 42 and 31.

Another fraction (0.35 g) of semi-solid white oil corresponded to 4-aminobutanol which was the main product.

Reaction of 3-Chloro-4-ethoxyformamidobutyl acetate (262) with Bases

A DBU

The substrate (262) (1.07 g, 4.51 mmol) was dissolved in chloroform (60 ml) and was stirred under a nitrogen atmosphere. DBU (1.37 g, 9.01 mmol) was added and the mixture was stirred for 75 h at room temperature. T.L.C. (silica gel) indicated that no reaction took place at all. The mixture was then heated under reflux for 17 h, but this did not change the outcome of the reaction. The starting material was recovered.

B Sodium hydride

To a stirred suspension of sodium hydride (0.06 g, 2.66 mmol) in DMF (10 ml) under nitrogen at room temperature was added (262) (0.63 g, 2.66 mmol) in DMF (5 ml). After stirring the mixture for 5 h, no reaction took place (T.L.C.). 0.06 g more of sodium hydride was added. After a further period of 10 h, no reaction took place. Eight more equivalents of sodium hydride were added. After stirring for 41 h, all the starting material had disappeared. The reaction was quenched with saturated solution of ammonium chloride (10 ml) and was extracted alternately with ether (2 x 2 ml) and dichloromethane (2 x 10 ml). The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to give 1.0 g of colourless oil which contained a lot of DMF. T.L.C. (silica gel) analysis showed the presence of at least 10 spots. No attempt was made to separate this mixture.

C Fluoride ion

(262) (1.70 g, 7.17 mmol) was dissolved in dry THF (20 ml) under nitrogen and was added to a suspension of caesium fluoride (2.18 g, 14.34 mmol) and benzyltriethylammonium chloride (0.16 g, 7.02 mmol) in THF (100 ml), also under a nitrogen atmosphere. The mixture was heated under reflux for 16 h. The solvent was evaporated *in vacuo* to leave a white powder, which was taken up in ethyl acetate-petroleum ether (10:20 v/v) and filtered through a pad of neutral alumina and the residue was washed well with the solvent. The filtrate was evaporated *in vacuo* to leave 0.52 g of colourless oil. Further washing with chloroform gave a white powder which was found to be the catalyst used. Analysis of the oil by T.L.C. indicated that it was a mixture of several products. The IR spectrum gave an absorption band at 1660 cm^{-1} (C=C).

The NMR spectrum of this oil did not indicate the presence of the desired product. No further separation of this mkxture was attempted.

Reaction of 4-Azido-3-iodobutyl acetate (259) with Triphenylphosphine

(259) (1.0 g, 3.53 mmol) was dissolved in dichloromethane (30 ml) at 0 °C. Triphenylphosphine (1.60 g, 6.11 mmol) was added and the mixture was stirred overnight at this temperature. The solvent was evaporated at reduced pressure and the residue was shaken with methanol (20 ml). The preciptated triphenylphosphine was filtered through a pad of ceilite. The filtrate was evaporated at reduced pressure to leave white crystals. T.L.C. analysis of these crystals revealed the presence of at least six spots. The NMR spectrum of these crystals indicated no presence of the desired product. Further treatment of these crystals with lithium aluminium hydride in THF at room temperature for 3 days gave a complex mixture from which no desired product could be isolated.

Attempted Oxidation of (2-Aziridinyl)ethanol (246)

A Fetizon Oxidation

To a stirred suspension of silver carbonate on celite (4.02 g, 23.91 mmol) in benzene (50 ml) was added (246) (0.26 g, 2.99 mmol) in chloroform (5 ml). The mixture was heated under reflux for 3 days. The inorganic salts were filtered through a pad of celite and the filtrate was evaporated at reduced pressure to leave 0.14 g of dark yellow oil. Analysis of this oil by T.L.C. on neutral alumina gave a streak. On silica gel, at least 8 spots were visible. The IR spectrum of this oil gave absorption bands at 1710 (C=O) and 1640 cm^{-1} (C=C). The NMR spectrum indicated that the aziridine ring was destroyed in the reaction.

B Potassium Permanganate

(246) (0.17 g, 1.95 mmol) was dissolved in wet acetone (20 ml). A slurry of potassium permanganate (0.62 g, 3.91 mmol) in acetone (30 ml) was added. The mixture was stirred at room temperature for 5 days. Acetone was evaporated *in vacuo*. The solid residue was diluted with water (10 ml) and filtered through celite. The filtrate was evaporated to leave white crystals (0.3 g). ν_{max} (Nujol) 3400 (NH) and 1560 cm^{-1} ($CO_2^-K^+$) Proton NMR spectrum of these crystals in D_2O only showed ring opened products. T.L.C. (silica gel, alumina) in methanol-water indicated a complex mixture of products.

Preparation of the Membrane and the Binding of [^{3}H]-Muscimol

This procedure is described by Jeffery *et al.* (in press).[46]

Supraoesophageal ganglia were removed from locust heads and homogenised in a Dounce glass homogeniser containing the following buffer: 50 mM morpholinopropane sulphonic acid (MOPS), 1 mM EGTA and 250 mM sucrose, pH 6.8, at a buffer to tissue ratio of 30:1. The homogenate was centrifuged at 300 g for 10 min, filtered through nylon bolting cloth (150 μm mesh) and the supernatant centrifuged at 100,000 g for 30 min. The pellet was re-suspended in 5 mM MOPS, pH 6.8, using a glass-glass homogensier, left at 4 °C for 15 min and then centrifuged at 100,000 g for 30 min. The pellet was re-suspended in 5 ml of extraction buffer and frozen at -20 °C overnight. The pellet was thawed at room temperature, centrifuged at 100,000 g for 30 min, re-suspended in 5 ml of extraction buffer and incubated at 25 °C for 15 min. The membranes were given a further 3 washes in an assay buffer 5 mM MOPS, 4 mM $MgCl_2$ and 0.15 mM choline chloride, all at pH 6.8; between each wash the membranes were centrifuged at 100,000 g for 30 min.

The binding of [^{3}H]-muscimol (29.4 Ci/mmol) (Amersham International) to the membranes was measured using a centrifugation assay. The pellets were solubilised overnight at room temperature with 75 µl of toluene. Binding was measured over the range of 1-100 mM [^{3}H]-muscimol in the presence and absence of 1 mM muscimol.

Isolation and Purification of GABA-T

This procedure is described by Jeffery *et al.* (in press).[46]

Locusts supplied by Larujon Locust Supplies, c/o Welsh Mountain Zoo, Colwyn Bay, North Wales, were decapitated and their *supraoesophageal ganglia* removed. The ganglia were disrupted in a glass/teflon homogensier containing the following buffer at a buffer to tissue ratio of 30:1 v/v: 50 mM MOPS, 300 mM sucrose, 2 mM $MgSO_4$, 3 mM EDTA, 0.1 mM phenylmethanesulphonyl fluoride and 5 mM dithiothrettol, all adjusted to pH 7.5. The homogenate was centrifuged at 800 x g for 10 min and the supernatant filtered through a nylon mesh (190 µm) and centrifuged at 10,000 x g for 30 min. The resulting crude mitochondrial pellet was re-suspended in 1-2 ml of the assay buffer, 50 mM *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine), pH 8.4, and used immediately. All extraction procedures were performed at 4 °C.

3 mg of GABASE were re-suspended in a mixture of 0.5 ml of 20 mM (1,3-bis[tris(hydroxymethyl)methylamino]bistrispropane), pH 7.3 and 0.5 ml of glycerol. A sample of 0.33 ml of this solution was applied to a pharmacia PD-10 column which had previously been equilibrated with 20 mM bistrispropane, pH 7.3. Initially, the protein was detected in the effluent by measuring column fractions at 280 nm; or a drop of dextran blue was added to the solution to facilitate protein detection. Dextran blue is eluted in the void volume and hence is used as a marker for protein eluting from the column. The total time needed for full elution was 2-3 min.

Inhibition of GABA-T

This was described by Jeffery *et al.* (in press).[46]

The compound under study was incubated with a known constant amount of enzyme in a constant volume of buffer: 50 mM Bicine, pH 8.5. At set time intervals the remainder of the assay constituents were added and the rate measured.

2-Ethylidene-2,2-dimethyl-1,3-dioxan-4,6-dione (307)

Method 1 To a stirred suspension of sodium hydride (0.25 g, 10.4 mmol) in THF (20 ml) at 0 °C under nitrogen was added Meldrum's acid (1.44 g, 10.0 mmol) in THF (20 ml) *via* a cannula. After stirring for 30 min at this temperature, acetaldehyde (1.1 ml, 20 mmol) was added dropwise *via* a syringe. The mixture was stirred for 2 h at 0 °C and then was allowed to warm to room temperature over 3 h. The solvent was removed at reduced pressure, room temperature, and the residue was diluted with saturated ammonium chloride solution, to which was added 5 ml 2N hydrochloric acid. The mixture was then extracted with dichloromethane (3 x 20 ml), washed the organic layer with water (20 ml), saturated brine (20 ml) and then dried ($MgSO_4$). The solvent was evaporated off *in vacuo* to yield a white crystalline solid. NMR and IR spectra only indicated the starting material.

Method 2 To a stirred solution of di-isopropylamine (1.4 ml, 0.01 mol) in THF (10 ml) at 0 °C under nitrogen was added dropwise *n*-butyl-lithium (6.9 ml, 0.01 mmol). After 30 min, the mixture was cooled to -78 °C and Meldrum's acid (1.44 g, 0.01 mol) in THF (10 ml) was then added *via* a cannula. After 30 min stirring of the mixture at -78 °C, acetaldehyde (0.56 ml, 0.01 mol) was added dropwise over 10 min and the reaction was stirred for a further 30 min. T.L.C. analysis, on silica gel, at this stage indicated a complex mixture of products. The reaction was quenched with saturated ammonium chloride (10 ml), extracted with ethyl

acetate (3 x 30 ml) and the organic layer was washed with water (20 ml), brine (20 ml) and dried ($MgSO_4$) to give, after removal of the solvent *in vacuo*, a yellow oil which was found to be a complex mixture of products.

Method 3* To a stirred dioxan (100 ml) at 0 °C under nitrogen was added titanium tetrachloride (3.8 ml) in carbon tetrachloride (15 ml). A yellow precipitate immediately formed. Meldrum's acid (5 g, 0.034 mol) in dioxan (10 ml) and acetaldehyde (1.96 ml, 0.034 mol) were added *via* different syringes. After stirring for 2 h, pyridine (5.6 ml) in dioxan (15 ml) was added dropwise over 15 min. The reaction was then stirred at this tmeperature for 20 h. Water (50 ml) and ether (50 ml) were added and the organic layer was separated. The aqueous layer was extracted with ether (5 x 50 ml). The combined organic layer was washed with water (50 ml), brine (50 ml), dried ($MgSO_4$) and evaporated off *in vacuo* to give a white solid which was crystallised from methylene chloride to give white crystals which corresponded to the Michael addition product (310). From the mother liquor was isolated a low melting white solid, not an oil, as reported.[10] ν_{max} ($CHCl_3$) 1630 (C=C) and 1740 (C=O), reported 1632 (C=C) and 1738 cm^{-1} (C=O), δ_H (60 MHz; $CDCl_3$) 1.7 (6H, s, CH_3), 2.45 (3H, d, CH_3), 7.8 (1H, q, HC=C), reported 8.08 (1H, q, CH=C), m/z 171 (M+1), 155 (M-15), reported 155 (M-15).

Attempted Oxidation Meldrum's Acid with Selenium Dioxide

Meldrum's acid (0.5 g, 3.47 mmol) and freshly sublimed selenium dioxide (0.39 g, 3.51 mmol) in dry dioxan (20 ml) were heated under reflux under nitrogen for 4 h. After work-up, only a complex mixture of products was obtained. No desired product was isolated from this mixture.

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*This procedure was described by Lehnert[14] for similar compounds.

2-Ethylidene-di-*t*-butyl malonate (306)

This compound was prepared according to the procedure of Lehnert,[14] as employed for compound (307). It was isolated in 28% yield. ν_{max} (thin film) 1640 (C=C) and 1730 cm^{-1} (C=O), δ_H (60 MHz; $CDCl_3$) 1.5 (18H, s, CH_3), 1.75 (3H, d, CH_3), and 6.75 (1H, q, CH=C), m/z 130 (M-112).

t-Butyl-4-bromo-2-*t*-butyloxycarbonylbut-2-enoate (311)

Compound (306) (0.12 g, 0.5 mmol), NBS (0.09 g, 0.5 mmol) and benzoylperoxide (0.01 g, 0.14 mmol) were heated under reflux for 40 min in carbon tetrachloride (5 ml). *N*-Succinimide was filtered and the solvent was removed at reduced pressure to yield 0.15 g yellow oil. Distillation gave a colourless oil. ν_{max} ($CHCl_3$) 1730 (C=O) and 1650 (C=C), δ_H (60 MHz; $CDCl_3$) 1.5 (18H, s, CH_3), 1.95 (2H, d, $BrCH_2CH$) and 6.8 (1H, t, CH=C).

Attemped Preparation of the Grignard Reagent of (311)

To a flame-dried magnesium turnings (0.47 mmol) under nitrogen was added THF (10 ml) and benzaldehyde (0.47 mmol). The mixture was stirred and the bromo compound (311) (0.47 mmol) was added dropwise. There was no change until the mixture was heated under reflux for 90 min, when all the metal dissolved. T.L.C. analysis of this mixture indicated the presence of unchanged benzaldehyde and the absence of the starting material. Quenching the mixture with ammonium chloride gave an oil, which was found to consist of benzaldehyde and several other components. No product was isolated form this mixture.

Attempted Alkylation of the Malonate (306)

To a solution of LDA (2.1 mmol) in THF (20 ml) at -78 °C under nitrogen was added HMPA (2.1 mmol). After stirring for 15 min, compound (306) (2.1 mmol) was added and the mixture was stirred for 30 min, followed by dropwise addition of benzyl bromide (2.1 mmol). After 30 min,

the mixture was allowed to warm to room temperature. T.L.C. analysis of the mixture at this stage revealed the presence of at least 8 components. After work-up only a complex mixture of products in which benzyl bromide was the main component, was isolated.

Methyl-6-chloro-5-oxo-2-trifluoroacetylaminohexanoate (314)

This compound was prepared according to the procedure of C. Smith,[7] as described below.

To a stirred solution of the acid chloride (292b)[8] (3.84 g, 10.33 mmol) in ether (20 ml) at 0 °C was added dropwise excess ice-cold ethereal diazomethane until no further evolution of gas was noted and the solution remained yellow in colour. Gaseous hydrogen chloride was bubbled through for 1 h at room temperature. The solvent was removed and the residue was columned on silica gel using ethyl acetate-petroleum ether as eluant to afford (314) in 60% yield as a white crystalline product, m.p. 80-81 °C, ν_{max} (Nujol) 3300 (NH), 1750 (C=O, ketone), 1735 (C=O, ester), and 1710 cm^{-1} (C=O, trifluoroacetyl), δ_H (60 MHz; $CDCl_3$) 7.4 (1H, br, s, NH), 3.7 (3H, s, $CO_2\underline{CH_3}$), 3.6 (2H, s, $\underline{CH_2}Cl$) and 2.3 (4H, m, $\underline{CH_2}\underline{CH_2}$), m/z 289 ($M^+$). [Found: C, 37.71; H, 3.84; N, 5.05%; $C_9H_{11}F_3NO_4Cl$ requires C, 37.32; H, 3.83; N, 4.84%].

Methyl-5-benzoyloxy-5-benzylcarbamoyl-6-chloro-2-trifluoroacetylaminohexanoate (293b)

This compound was prepared according to the procedure of C. Smith,[7] as described below.

Benzyl isocyanide (0.17 ml, 1.4 mmol), benzoic acid (0.17 g, 1.4 mmol) and the ketone (314) (0.4 g, 1.4 mmol) were stirred at room temperature for 20 h. The mixture was subjected to column chromatography on silica gel to give a yellow oil. ν_{max} (thin film) 3320 (NH), 1730 (C=O, ester)

and 1680 cm^{-1} (C=O, amide), δ_H (60 MHz; $CDCl_3$) 7.85 (1H, br, NH), 7.75 (1H, d, $\underline{NH}CH_2Ph$), 7.35 (5H, s, $\underline{Ph}CO_2$), 7.15 (5H, s, $\underline{Ph}CH_2$), 4.45 (2H, d, $PhC\underline{H_2}$), 3.65 (3H, s, $CO_2C\underline{H_3}$), 3.55 (2H, s, $C\underline{H_2}Cl$), 2.3 (4H, m, $C\underline{H_2}C\underline{H_2}$) and 4.18 (1H, m, CH), m/z 529 (M+1).

Methyl-5-acetoxy-5-benzylcarbamoyl-6-chloro-2-trifluoroacetyl-aminohexanoate (293a)

This compound was also prepared from (314), acetic acid and benzyl isocyanide, according to the procedure of C. Smith[7] and was isolated in 58% yield. ν_{max} (thin film) 3300 (NH), 1720 (C=O) and 1665 cm^{-1} (C=O, amide), δ_H (60 MHz; $CDCl_3$) 7.4 (1H, br, NH), 7.05 (5H, s, $\underline{Ph}CH_2$), 4.3 (2H, d, $PhC\underline{H_2}$), 3.65 (3H, s, $CO_2C\underline{H_3}$), 2.05 (3H, s, $C\underline{H_3}CO$), 3.50 (2H, s, $C\underline{H_2}Cl$) and 1.9 (4H, m, CH_2CH_2), m/z 467 (M+1).

Preparation of Methyl-4-(1-*N*-benzyl-3-benzoyloxyazetidinyl)-2-trifluoroacetylaminobutanoate (294b)

This compound was prepared according to Smith's procedure.[7] The chloroamide (293b) (1.4 g, 2.65 mmol), caesium fluoride (2.01 g, 13.26 mmol) benzyl triethylammonium bromide (0.18 g, 0.67 mmol) in THF (20 ml) under nitrogen were heated under reflux for 16 h. After removal of the solvent at reduced pressure, the residue was subjected to column chromatography on silica gel eluting with ethyl acetate-petroleum ether to get 0.18 g (62%) of colourless oily product which solidified on standing. ν_{max} (thin film) 3400 (NH) and 1720-50 cm^{-1} (C=O). The 60 MHz δ_H NMR spectrum corresponded to structure (294b). The mass spectrum gave m/z 493 (M+1).

Preparation of Methyl-4-(1-*N*-benzyl-3-acetoxyazetidinyl)-2-trifluoroacetylaminobutanoate (294a)

The preparation of this compound was identical to that of (294b) and was isolated in 36% yield. ν_{max} ($CHCl_3$) 3400 (NH) and 1720-50 cm^{-1} (C=O, br), δ_H (60 MHz; $CDCl_3$) 7.4 (1H, NH, br), 7.05 (5H, s, $\underline{Ph}CH_2$), 4.3 (2H, d

$PhCH_2$), 3.65 (3H, s, CO_2CH_3), 2.05 (3H, s, CH_3CO_2), 1.90 (4H, m, CH_2CH_2) and 3.2 (2H, CH_2-N), m/z 430 (M^+).

Preparation of 3,4-Dimethoxybenzyl Isocyanide

3,4-Dimethoxybenzylamine (5 g, 0.03 mol), benzyltriethylammonium bromide (9 mg), dichloromethane (8.9 ml), chloroform (2.4 ml) and 30% sodium hydroxide (8.9 ml) were heated under reflux for 30 min. Water (15 ml) was added and the organic layer was extracted into dichloromethane (3 x 20 ml), washed with brine (20 ml) and dried ($MgSO_4$) to give a brown oil which was purified by column chromatography on silica gel using ethyl acetate-petrolum ether as eluant to yield 2.21 g (42%) of colourless oil. ν_{max} (thin film) 2140 cm^{-1} (N≡C) and 1580 cm^{-1} (aromatic), δ_H (100 MHz; $CDCl_3$) 3.81 (6H, s, 2 x OCH_3), 4.44 (2H, s, CH_2Ph) and 6.80 (3H, s, aromatic), δ_C (100 MHz; $CDCl_3$) 45.20 (CH_2Ph), 55.96 (2 x OCH_3), 110.14, 110.57, 111.49, 119.24, 149.14 and 148.41 (aromatic carbons), m/z 177 (M^+) and 162 (M-15).

Preparation of Methyl-5-chloroacetoxy-6-chloro-5(3,4-dimethoxybenzyl)carbamoyl-2-trifluoroacetylaminohexanoate (318)

The ketone (314) (3.92 g, 13.56 mmol) and chloroacetic acid (1.28 g, 13.62 mmol) were dissolved in dichloromethane (20 ml). 3,4-Dimethoxybenzyl isocyanide (2.21 g, 12.49 mmol) was then added. The mixture was heated under reflux for 20 h under a nitrogen atmosphere. The solvent was removed *in vacuo* and the residue was purified by dry-column chromatography on silica gel eluting with ethyl acetate-petroleum ether to give 3.99 g (53%) of crystalline product. ν_{max} ($CHCl_3$) 3410 (NH), 1730 (C=O, ester) and 1670 cm^{-1} (C=O, amide), δ_H (100 MHz; $CDCl_3$) 2.08 (4H, m, CH_2CH_2), 3.70 (2H, s, CH_2Cl), 3.81 (9H, s, 3 x OCH_3), 4.05 (2H, s, $ClCH_2CO_2$), 4.45 (2H, d, CH_2Ph), 4.24 (1H, m, CH-CO_2CH_3), 6.80 (3H, s, aromatics), 7.08 (1H, d, br, NH) and 7.44 (1H, t, br, NH), δ_C (100 MHz; $CDCl_3$) 45.40 (CH_2Ph), 55.91 (3 x OCH_3), 111.06, 111.44, 119.94 (3 x CH aromatics), 149.36, 148.71

(aromatics), 53.14 (C2), 40.98 (C6), 25.25 (C4), 28.49 (C3), 60.46 ($Cl\underline{C}H_2CO_2$), 170.38 ($\underline{C}O_2$), 168.10 (NH$\underline{C}$O), 164.85 ($\underline{C}OCF_3$) and 130.02 ($CO\underline{C}F_3$), m/z 561 (M+1), 563 (M+3), 255 (M-HCl), 491 (M-70). [Found: C, 44.84; H, 4.47%; N, 4.84%. $C_{21}H_{25}O_8N_2F_3Cl_2$ requires C, 44.93; H, 4.49; N, 4.99%, M, 561.3377].

Attempted Cyclisation of Methyl-5-chloroacetoxy-6-chloro-5(3,4-dimethoxybenzyl)carbamoyl-2-trifluoroactylaminohexanoate (318)

Method 1 The chloro- amide (318) (0.35 g, 0.625 mmol), anhydrous caesium floride (0.95 g, 6.25 mmol) and benzytriethylammonium bromide (0.085 g, 0.313 mmol) were heated under reflux with stirring in THF (50 ml) for 8 h under a nitrogen atmosphere. The solvent was evaporated off *in vacuo* and the residue was taken up in chloroform, filtered off through celite and evaporated to give a gummy oil which was found to consist of nine spots on T.L.C. No desired product was isolated from this mixture.

Method 2* To a stirred suspension of sodium hydride (0.84 g, 35 mmol) in DMF (20 ml) under a nitrogen atmosphere was added the amide (318) (0.36 g, 0.64 mmol) in dichloromethane (20 ml). The mixture was stirred at 80 °C for 5 h, cooled to room temperature, filtered and diluted with a saturated solution of ammonium chloride (20 ml). The mixture was then extracted with dichloromethane (3 x 20 ml) and dried ($MgSO_4$) to give a brown oil which was found to be a complex mixture of products.

Method 3 To a suspension of neutral alumina type 1 (0.3 g) and powdered potassium hydroxide (0.1 g, 1.79 mmol) in dioxane (10 ml) was added dropwise the amide (318) (0.13 g, 0.23 mmol) under nitrogen. The

*The same complex mixture was obtained when the reaction was conducted in THF at -78 °C to room temperature under a nitrogen atmosphere.

mixture was stirred at 80 °C for 17 h. The solvent was evaporated and a crystalline residue was shaken with ethyl acetate, filtered and the filtrate was evaporated *in vacuo*, but contained no product.

Attempted hydrolysis of Methyl-4-(1-*N*-benzyl-3-benzoyloxyazetidinyl)-2-trifluoroacetylaminobutanoate (294b)

Method 1 Sodium metal (0.0137 g, 0.60 mmol) was dissolved in methanol (2 ml) under a nitrogen atmosphere and added to a stirred solution of the ester (294b) (0.20 g, 0.4 mmol) in methanol (5 ml) at room temperature. The mixture was stirred for 7 h, evaporated *in vacuo* and subjected to column chromatography on silica gel using ethyl acetate-petroleum ether to afford a colourless oil (55 mg, 34%). ν_{max} 3300 (NH, br), 1730 (C=O, ester), 1690 cm^{-1} (C=O, amide) and 1750 cm^{-1} (C=O, lactam), m/z 389 (M+1), 370 ($M-H_2O$) corresponding to methyl-4-(1-*N*-benzyl-3-hydroxyazetidinyl)-2-trifluoroacetylaminobutanoate* (315).

Method 2 The ester (294b) (0.2 g, 0.41 mmol) and iodotrimethylsilane† (0.08 g, 0.41 mmol) in acetonitrile (10 ml) were stirred for 20 h under reflux. The mixture was then cooled to room temperature and was diluted with water (10 ml). The mixture was extracted with chloroform (3 x 10 ml). The combined organic layer was washed once with 5% sodium thiosulphate solution and dried (Na_2SO_4) to give, after evaporation of the solvent, a starting material.

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*This compound has been prepared by C. Smith.[7]

†Iodotrimethylsilane was generated *in situ* from dry sodium iodide and dry chlorotrimethylsilane.

Attempted Hydrolysis of Methyl-4-(1-*N*-benzyl-3-hydroxyazetidinyl)-2-trifluoroacetylaminobutanoate (315)

The ester (315) (0.11 g, 0.28 mmol) was dissolved in methanol (1.2 ml) and aqueous 10% sodium hydroxide (2.9 ml) was added. The mixture was heated under reflux for 1 h and the solvent was removed *in vacuo*. Analysis of the residue by T.L.C. on silica gel using methanol-water as eluent indicated a mixture of several components. After acidification of the mixture with 2N hydrochloric acid and extraction into ethyl acetate gave an oil which was found to contain at least 9 spots according to T.L.C. Potassium hydroxide gave the same result.

Atempted Hydrogenolysis of the Benzyl Group in (294b)*

(294b) (0.55 g, 1.12 mmol) and palladium chloride (0.03 g, 0.15 mmol) were hydrogenated in acetic acid (10 ml) 20 h at room temperature at atmospheric pressure. The catalyst was filtered through celite and the solvent was evaporated *in vacuo* to leave the unreacted starting material. Increasing the hydrogen pressure to 150-200 psi gave an intractable mixture.

Reaction of Methyl-5-chloroacetoxy-6-chloro-5-(3,4-dimethoxybenzyl)-carbamoyl-2-trifluoroacetylaminohexanoate (318) with Nucleophiles

A The chloroacetate (318) (0.92 g, 1.64 mmol) and thiourea (0.14 g, 1.84 mmol) in ethanol (23 ml) and pyridine (23 ml) were heated under reflux for 2 h. The solvents were removed at reduced pressure and the residue was taken up in ethyl acetate (10 ml) and filtered through celite. The filtrate was evaporated *in vacuo* to leave a brown oil. T.L.C. analysis of this mixture indicated at least six products. Column chromatography on silica gel using ethyl acetate-methanol gave 80 mg of brown gum.

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*Catalytic transfer hydrogenation on Pd/C and ammonium formate in refluxing methanol gave no reaction.

ν_{max} ($CHCl_3$) 3400 (NH, OH, br), 1730 (C=O, ester) and 1680 cm^{-1} (C=O, amide), δ_H (60 MHz; $CDCl_3$) 2.0 (4H, m, $C\underline{H}_2C\underline{H}_2$), 3.80 (2H, $C\underline{H}_2Cl$), 3.90 (9H, s, 3 x OCH_3), 4.40 (2H, $\underline{CH}_2Ph$), 4.20 (1H, m, $\underline{C}HCO_2$) and 6.90 [3H, s, $CH_2C_6\underline{H}_3(OCH_3)_2$], m/z 484 ($M^+$) and 448 (M-HCl) corresponding to methyl-6-chloro-5-(3,4-dimethoxybenzyl)carbamoyl-5-hydroxy-2-trifluoroacetyl-aminohexanoate (319) which was found to be unstable on standing.

B (318) (0.18 g, 0.32 mmol) was dissolved in methanol (6 ml) and sodium acetate (0.1 g, 1.27 mmol) dissolved in methanol-water (2 ml) (1:1) was added. The mixture was heated under reflux for 80 min and the solvent was evaporated at reduced pressure. The residue was dissolved in 2N hydrochloric acid (10 ml) for 30 min at 80 °C. Saturated solution of sodium chloride was added and the mixture was extracted with ethyl acetate (5 x 20 ml). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to give 100 mg of colourless gum. Column chromatography of this on silica gel gave 28 mg of product corresponding to the alcohol (319).

C (318) (0.27 g, 0.48 mmol) and lithium hydroxide (0.42 g, 10.0 mmol) were suspended in methanol (12 ml) and heated under reflux for 13 h. Evaporation of the solvent left a white solid which was dissolved in 2N hydrochloric acid (5 ml) and extracted with ethyl acetate (3 x 5 ml). The organic layer was dried (Na_2SO_4) and evaporated to leave no product. The aqueous layer was freeze-dried and the solid residue triturated with ethyl acetate to get an oil which was found to be a complex mixture of products.

Attempted Cleavage of the Benzyl Group of (318)*[28]

(318) (0.23 g, 0.41 mmol), potassium persulphate (0.44 g, 1.63 mmol) and di-sodium hydrogen phosphate dihydrate (0.146 g, 0.82 mmol) were suspended in acetonitrile (15 ml). Water (10 ml) was then added. The

*Catalytic hydrogenation on palladium on carbon in methanol gave no reaction.

mixture was stirred at 80 °C for 2 h. The solvents were removed at reduced pressure and the residue was extracted with hot absolute ethanol (3 x 25 ml). Ethanol was evaporated *in vacuo* to afford a faint green gum which was found to consist of 9 spots by T.L.C. analysis.

Preparation of (S)-Methyl-6-dimethoxyphosphoryl-5-oxo-2-trifluoroacetylaminohexanoate (321)

Method 1 (314) (0.32 g, 1.1 mmol) and trimethyl phosphite (10 ml) were heated under reflux for 5 h. Excess trimethyl phosphite was removed at reduced pressure. The residue was subjected to column chromatography on silica gel to afford 0.18 g (45%) of colourless oil. ν_{max} (thin film) 3250 (NH), 1750 (C=O, ketone), 1730 (C=O, ester), 1660 (C=O, amide), 1270 (P=O) and 1050 cm^{-1} (P-OCH_3), δ_H (100 MHz; $CDCl_3$) 2.20 (4H, m, $\underline{CH_2}CH_2$), 3.79 (6H, d, J* = 5 Hz, 2 x OCH_3), 3.84 (3H, s, $CO_2\underline{CH_3}$), 4.60 (2H, m, $CO\underline{CH_2}P$), 4.80 (1H, m, CH) and 8.05 (1H, br, NH), m/z 364 (M+1) [+] ion FAB and 362 (M-1) [-] ion FAB.

Method 2† To a stirred solution of di-isopropylamine (1.42 ml, 9.99 mmol) in THF (20 ml) at 0 °C under a nitrogen atmosphere was added dropwise *n*-butyl-lithium (6.24 ml, 9.99 mmol) *via* a syringe. After stirring the mixture for 30 min it was cooled to -78 °C and dimethyl methylphosphonate (1.08 ml, 10.08 mmol) at -78 °C was added dropwise *via* a cannula. The mixture was stirred at this temperature for 2 h and was then warmed to 0 °C, held for 2 h and then was allowed to warm to room temperature overnight. Saturated solution of ammonium chloride (30 ml) was added and the mixture was extracted with ethyl acetate (3 x 50 ml). The combined organic layer was washed once with water (50 ml), brine (20 ml) and then dried ($MgSO_4$). After evaporation of the solvent *in vacuo* 0.26 g of brown oil was obtained. T.L.C. analysis revealed at least 6 spots. Column chromatographic

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*J refers to J_{P-O-CH}.

†Compound (292b) (0.5 equiv.) was added 30 min after addition of dimethyl methylphosphonate.

separation of this mixture on silica gel gave 50 mg of colourless oil. ν_{max} ($CHCl_3$) 3360 (NH), 1730 (C=O), 1750 (C=O) and 1670 cm^{-1} (C=O, amide), m/z 364 (NH) and 284 (M-79, $CH_3O_2\overset{+}{P}H$) corresponding to the phosphonate (321).

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